SYNTHESIS, CHEMICAL, BIOCHEMICAL, X-RAY AND OTHER SPECTRAL STUDIES OF MODIFIED STEROIDS

RESUME

THESIS SUBMITTED FOR THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

Mrs. SHEEBA SHAFI

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

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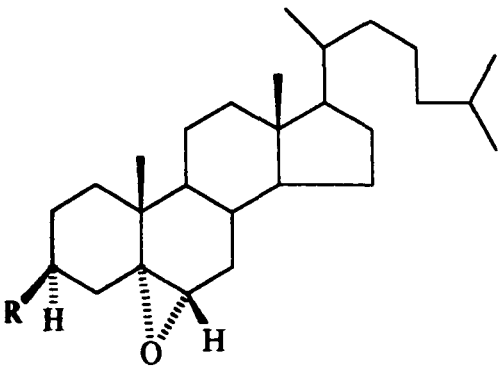
RESUME

The chemistry of steroids is a matter of great interest because of their immense use in research and industry owing to their broad spectrum of biological properties. In the thesis, the synthesis of some important hetero steroids are described. The compounds synthesized were characterized on the basis of chemical, analytical and spectral evidences. Biochemical studies of some of them has been done. The results were summarized chapterwise as below.

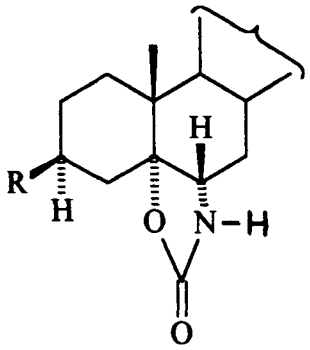
CHAPTER-ONE

Synthesis of Steroidal Oxazolines and Aziridines (Reaction of Steroidal Epoxides) :

Reaction of steroidal epoxides with appropriate reagents leading to the synthesis, of steroidal oxazolidines, oxazoles, oxazolines, oxathiolanethiones and aziridine have been reported from our laboratories and from other research centres because of their pharmaceutical importance which include inflammatory, hypertensive, tranquilizing and carcinostatic activities. Recent publication from our laboratories deals with the synthesis of steroidal oxazolidinones (IV-VI) which involved reactions of steroidal epoxides (I-III) with glycine.

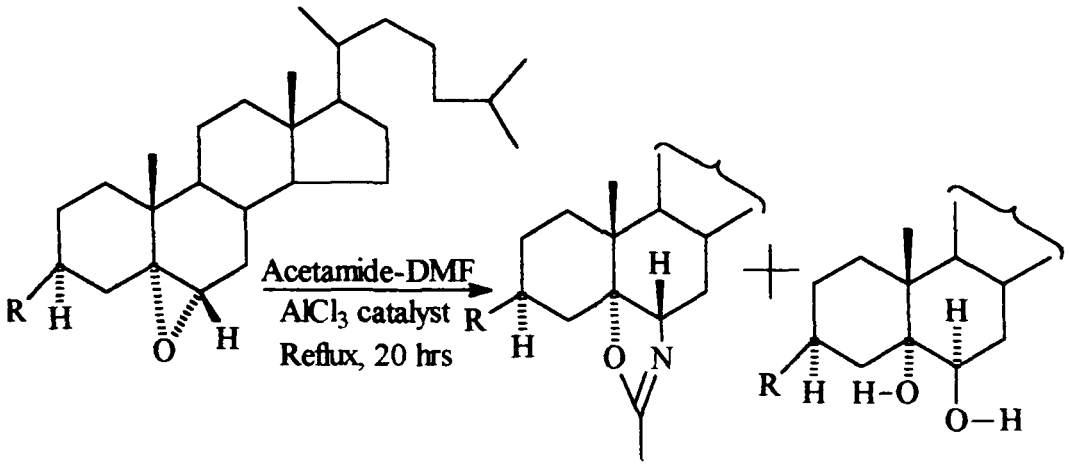


R	
(I)	H
(II)	Cl
(III)	OAc



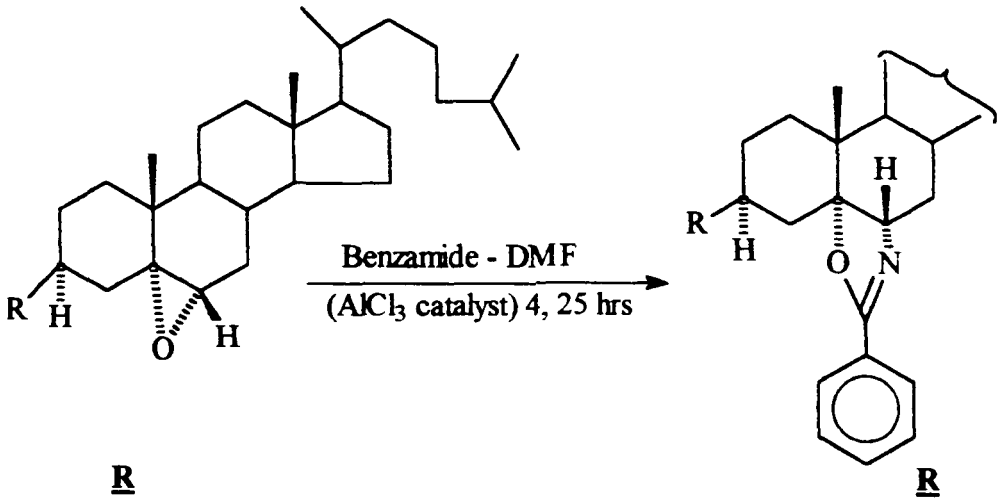
R	
(IV)	H
(V)	Cl
(VI)	OAc

In continuation to our study related to the synthesis of steroidal compounds containing heterocyclic ring, new steroidal oxazolines and aziridines in cholestane series were reported in this chapter, when 5,6α-epoxy-5α-cholestane (I), its 3β-chloro (II) and 3β-acetoxy (III) analogues were treated with actetamide in DMF ($AlCl_3$ as catalyst) at reflux condition for 20-25 hrs. afforded 5α-cholestano[5,6α-d]-2'-methyl-2-oxazoline (VII), its 3β-chloro (VIII) and 3β-acetoxy (IX) analogues along with 5,6β-dihydroxy-5α-cholestane (X), its 3β-chloro (XI) and 3β-acetoxy (XII) analogues.



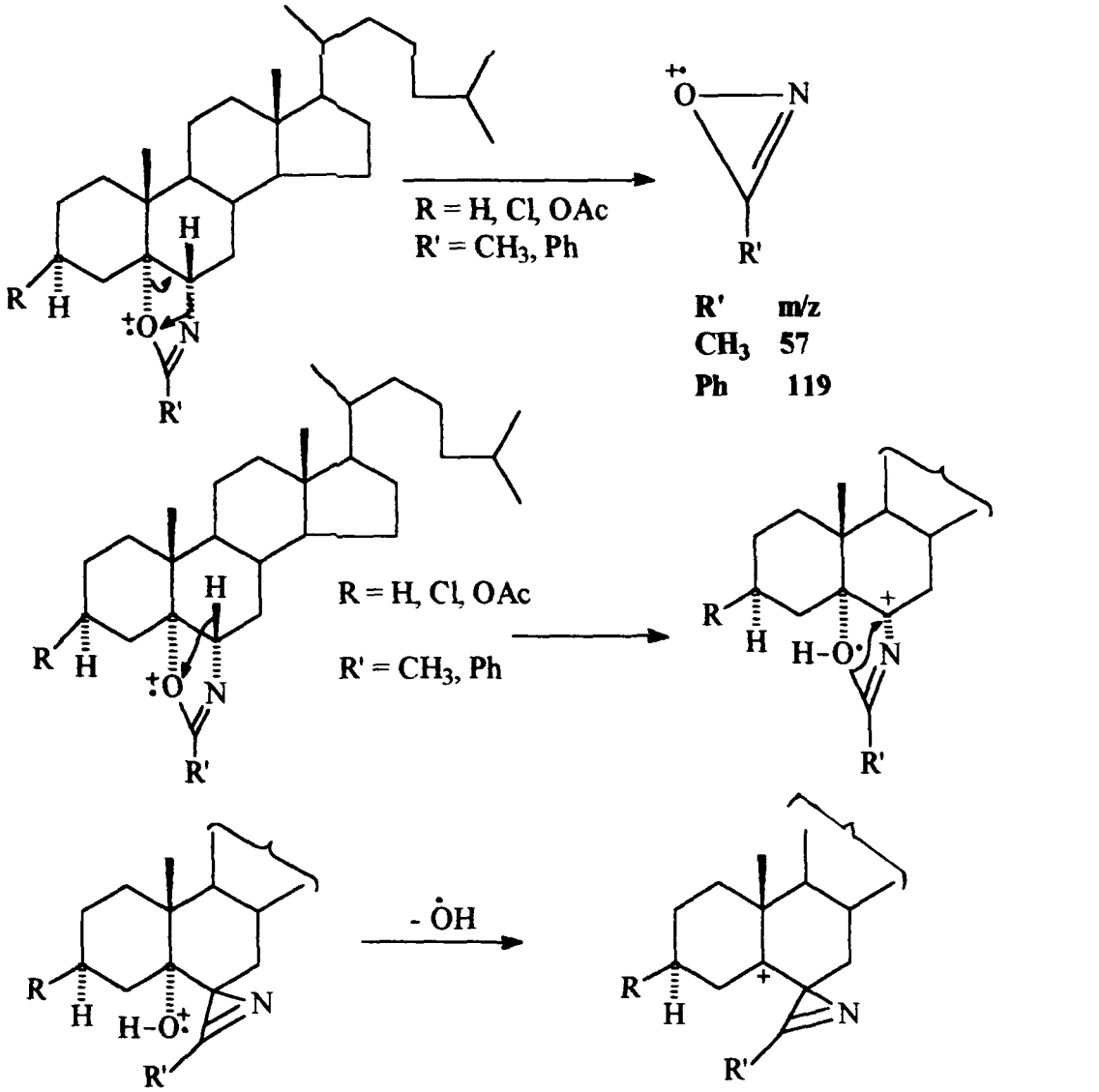
R	R	R
(I) H	(VII) H	(X) H
(II) Cl	(VIII) Cl	(XI) Cl
(III) OAc	(IX) OAc	(XII) OAc

When the same reaction was repeated with benzamide – DMF ($AlCl_3$ a catalyst, reflux for 25 hrs.) with steroidal epoxides (I – III), 5α-cholestano [5, 6α-d]-2′-phenyl-2-oxazoline (XIII) its 3β-chloro (XIV) and acetoxy (XV) analogues were obtained along with the steroidal diols (X – XII).



R	R
(I) H	(XIII) H
(II) Cl	(XIV) Cl
(III) OAc	(XV) OAc

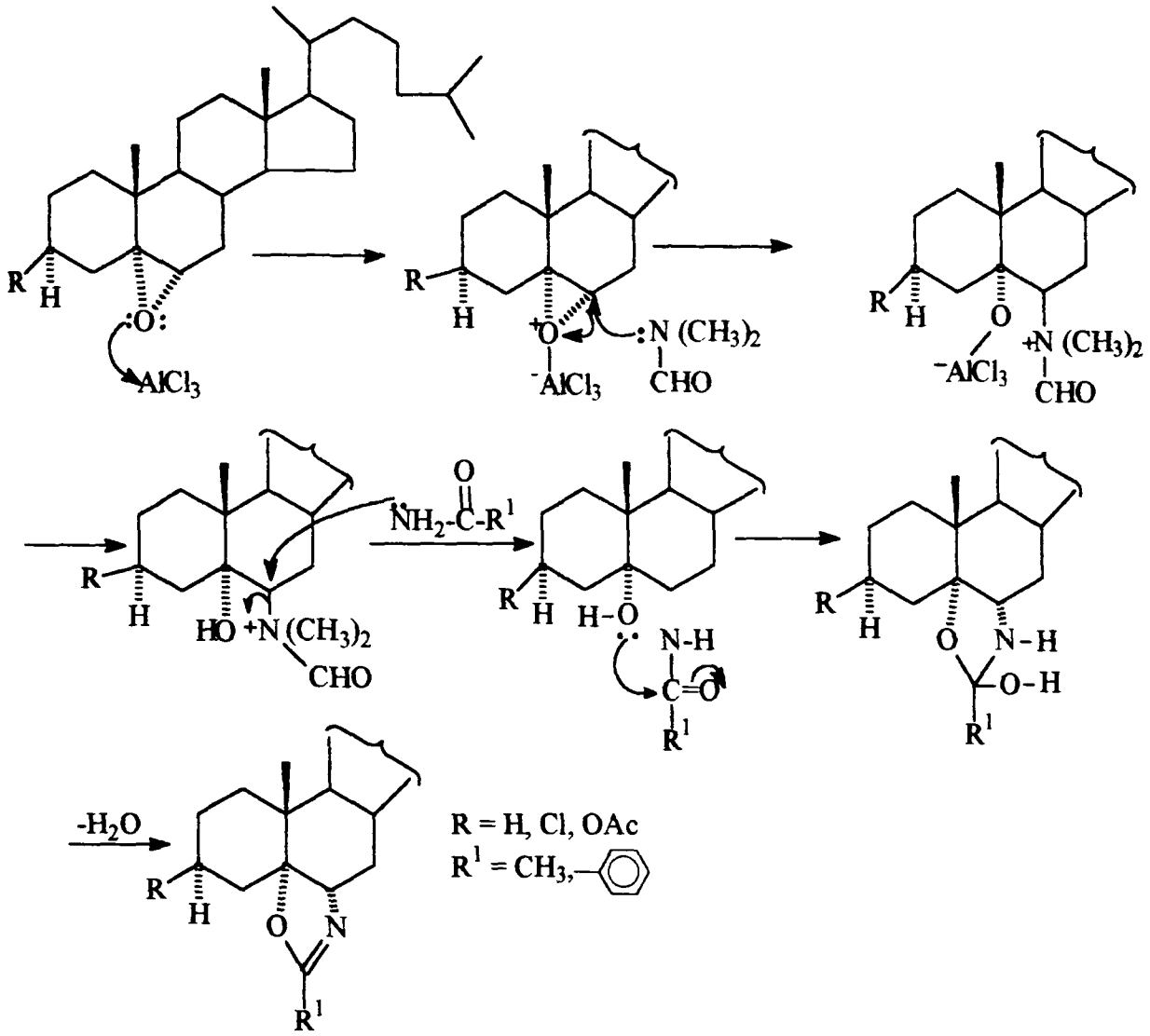
The structure of steroidal oxazolines (VII – IX) and (XIII – XV) was established on the basis of analytical and spectral evidences. Mass spectral studies has given strong support for the assigned structure. Two diagnostic fragment ions obtained were given in scheme – 1.



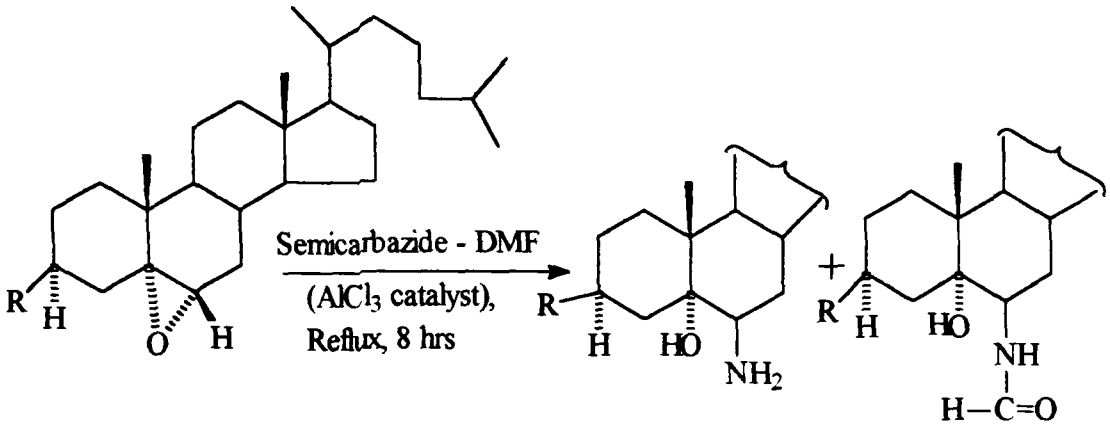
R	R'	M/z	R	R'	m/z
H	CH_3	410	H	Ph	460
Cl	CH_3	444 / 446	Cl	Ph	506 / 508
OAc	CH_3	457	OAc	Ph	530

Scheme - 1

A mechanism has been written to explain the formation of steroidal oxazolines (VII – IX) and (XIII – XV) in which double SN^2 – inversion on epoxide ring carbon (C6) has occurred. Formation of diols (X – XII) was because of the trans diaxial ring opening of epoxides (I – III).



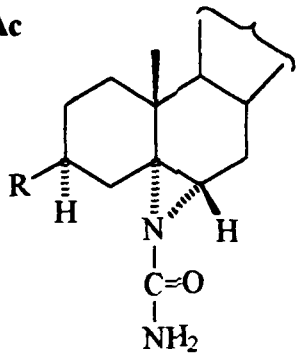
When 5, 6α-epoxy-5α-cholestane (I), its 3β-chloro (II) and 3β-acetoxy (III) were treated with semicarbazide in DMF ($AlCl_3$ as catalyst) afforded 5-hydroxy-6β-amino-5α-cholestane (XVI), 5-hydroxy-6β-amino-N-formyl-5α-cholestane (XIX) and N-amido-5α-cholestano [5, 6-b]-aziridine (XXII) and their corresponding 3β-chloro (XVII, XX, XXIII) and acetoxy (XVIII, XXI, XXIV) analogues.



R	
(I)	H
(II)	Cl
(III)	OAc

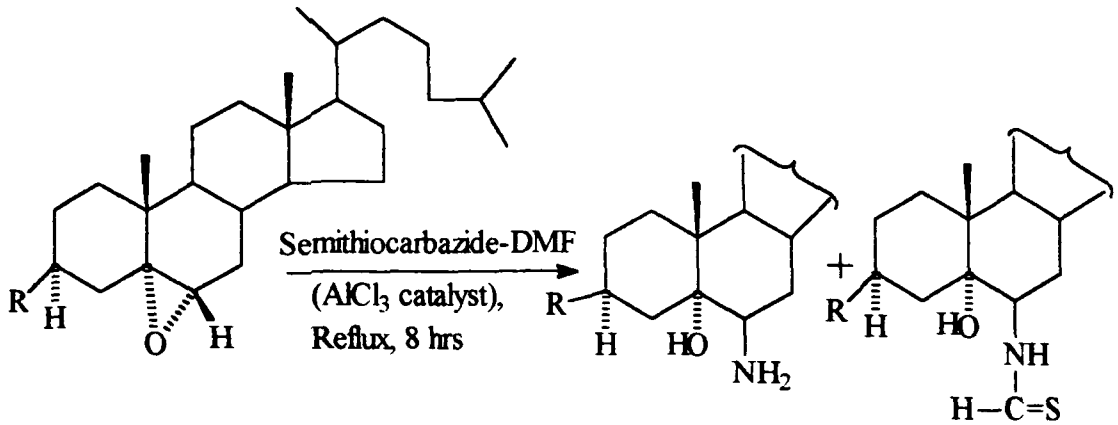
R	
(XVI)	H
(XVII)	Cl
(XVIII)	OAc

R	
(XIX)	H
(XX)	Cl
(XXI)	OAc



R	
(XXII)	H
(XXIII)	Cl
(XXIV)	OAc

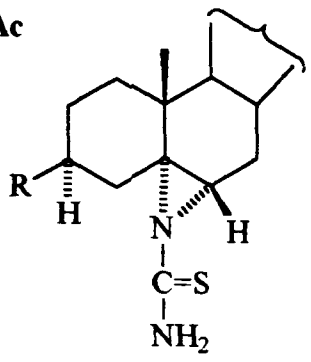
When the same epoxides (I–III) were treated with phenyl semicarbazide under identical reaction conditions same products (XVI – XVIII). (XIX – XXI) and (XXII – XXIV) were obtained. The steroidal epoxides (I – III) when treated with semithiocarbazide under same reaction conditions afforded hydroxyamino compounds (XVI – XVIII), aminothioformyl compounds (XXV- XXVII) and thioamido aziridines (XXVIII – XXX).



	R
(I)	H
(II)	Cl
(III)	OAc

	R
(XVI)	H
(XVII)	Cl
(XVIII)	OAc

	R
(XXV)	H
(XXVI)	Cl
(XXVII)	OAc

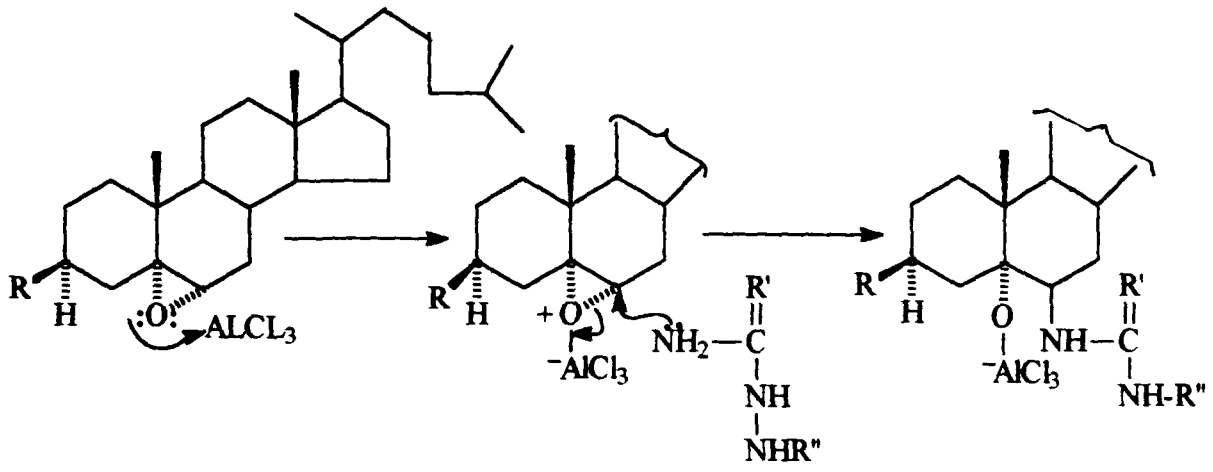


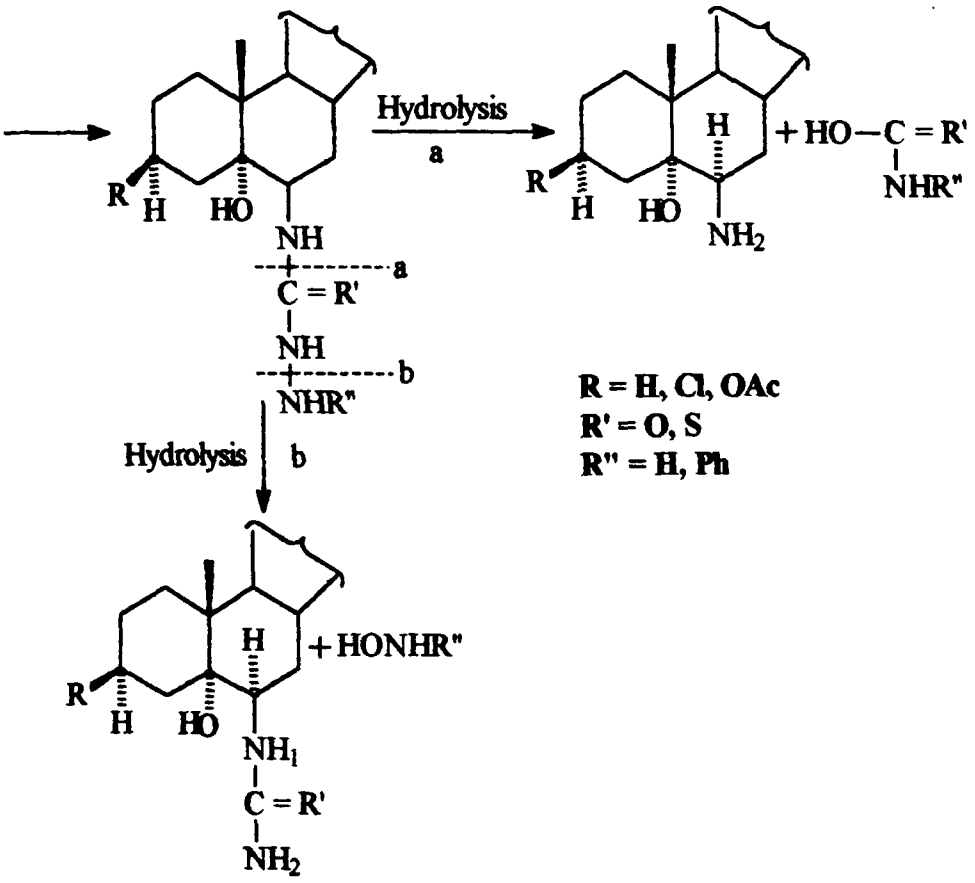
	R
(XXVIII)	H
(XXIX)	Cl
(XXX)	OAc

The structure of these amino steroids was confirmed on the basis of analytical and spectral (IR, ^{1}H-NMR and Mass) evidences. Formation of these compounds was explained on the basis of mechanism (1 and 2).

Mechanism – 1 :

Formation of hydroxy amino compounds and amino formyl and thio formyl compounds were explained as follows where during hydrolysis via path a provided hydroxyamino compounds where as path b afforded amino formyl and thio formyl compounds.

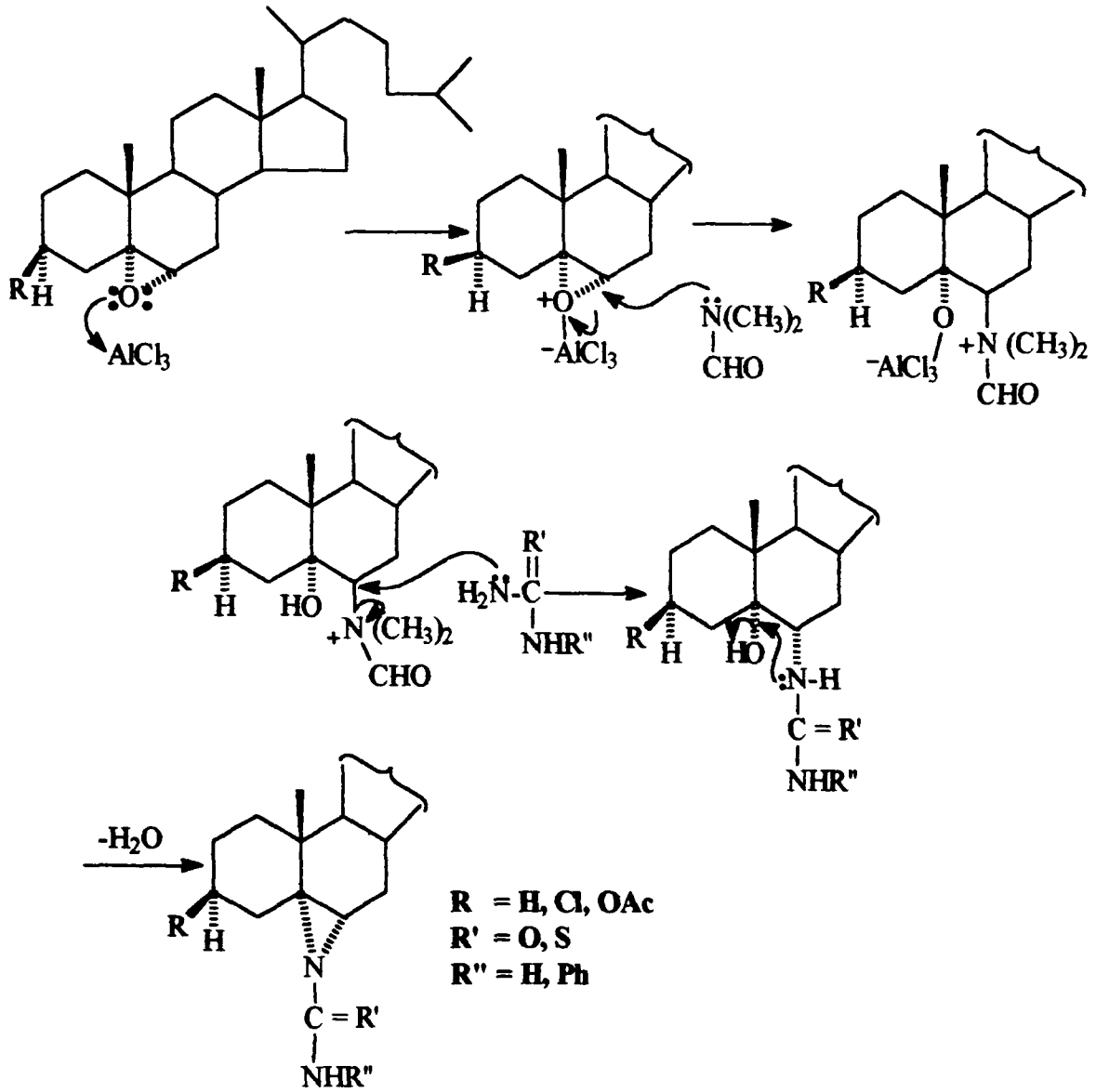




Formation of amidoaziridines and thioamidoaziridines can be explained on the basis of mechanism – 2. In which double SN^2 – inversion on epoxide ring carbon (C6) has occurred.

x

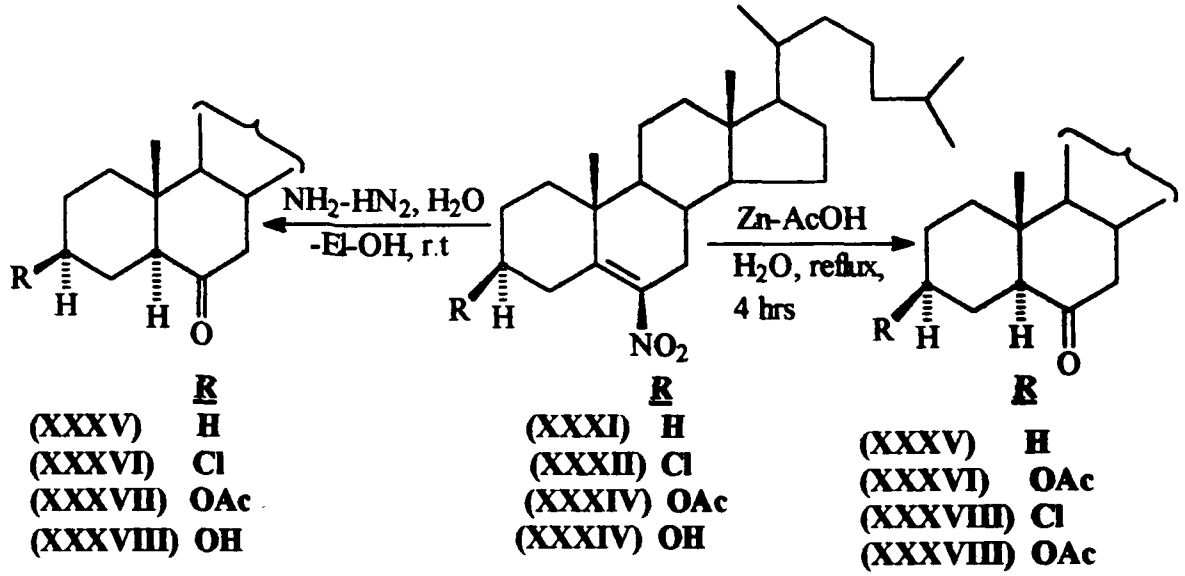
Mechanism – 2 :



CHAPTER-TWO

Reduction of Vinyl Nitrosteroids :

In the recent past synthesis of steroidal compounds have gained importance because of biological activities associated with them. Reduction is one among the various reactions used in the synthetic pathway leading to important steroidal compounds. Many types of reagents which have been successfully employed for reduction are hydrogen with metal, lithium alumininum hydride, zinc acetic acid, sodium borohydride and other metallic hydrides. Photochemical and electrochemical methods were also employed for reduction. Raney Nickel catalysed hydrazine - hydrate reduction has not been studies thoroughly. In this chapter a comparative study has been made between zinc–acetic acid and hydrazinc-hydrate (Raney Nickel catalysed) reduction of vinyl nitrosteroids. 6-nitrocholest-5-ene (XXXI), its 3β-acetoxy (XXXII), 3β-chloro (XXXIII) and 3β-hydroxy (XXXIV) analogues were subjected to both zinc-acetic acid and hydrazine-hydrate (Raney Nickel) catalysed reduction.

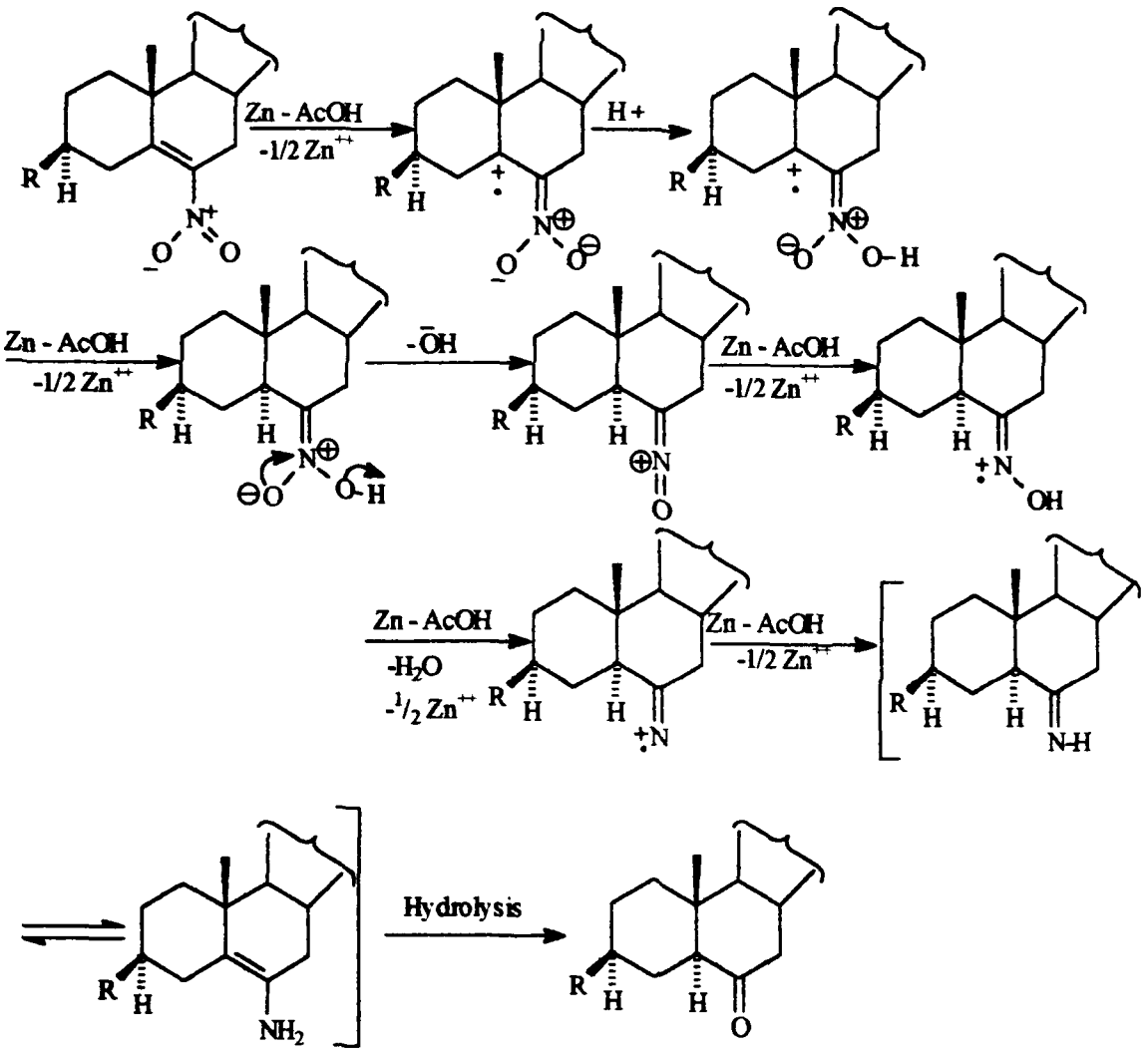


R	R	R
(XXXV) H	(XXXI) H	(XXXV) H
(XXXVI) Cl	(XXXII) Cl	(XXXVI) OAc
(XXXVII) OAc	(XXXIV) OAc	(XXXVIII) Cl
(XXXVIII) OH	(XXXIV) OH	(XXXVIII) OAc

Both the reduction methods gave the same ketones with respect to the vinyl nitrosteroids but the reaction conditions are mild and yield is better in hydrazinc-hydrate (Raney Nickel) catalysed reduction. The mechanism of reduction is proposed in both the cases.

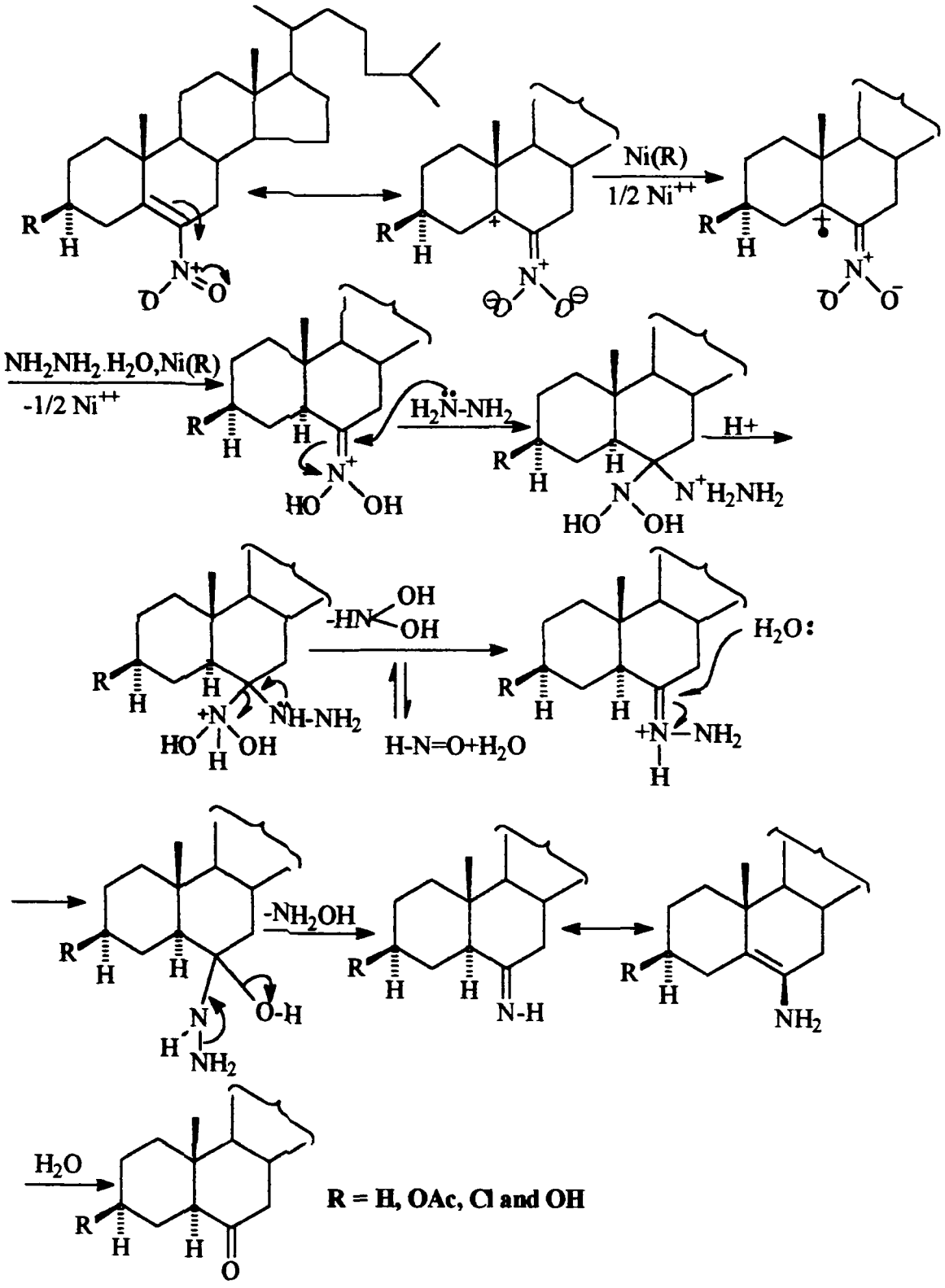
Zn-AcOH Reduction of Vinylnitro Steroids :

Mechanism :



Hydrazine – Hydrate (Raney Nickel Catalysed) Reduction :

Mechanism


Ni(R)
1/2 Ni++
NH2NH2.H2O,Ni(R)
-1/2 Ni++
H2N-NH2
H+
-HN(OH)OH
H2O:
H-N=O+H2O
-NH2OH
H2O
R = H, OAc, Cl and OH

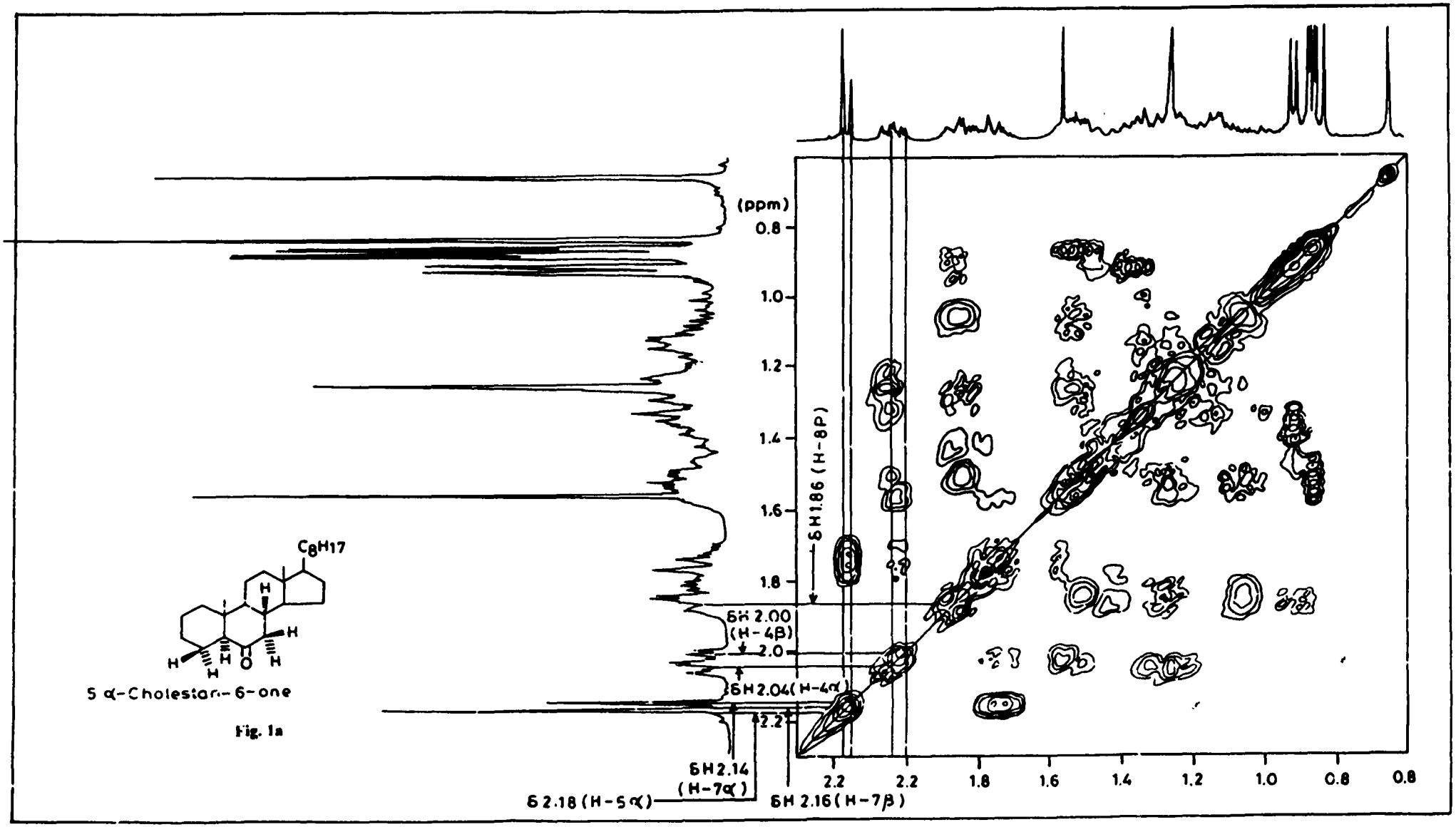

(ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
δH 1.86 (H-8β)
δH 2.00 (H-4β)
δH 2.04 (H-4α)
δH 2.14 (H-7α)
δ 2.18 (H-5α)
δH 2.16 (H-7β)
2.2
2.2
1.8
1.6
1.4
1.2
1.0
0.8
0.8
C8H17
H
H
H
H
H
O
5 α-Cholestan-6-one

Fig. 1a

Since the total transformation of vinylnitrosteroidal to (XXXI – XXXIV) to ketones (XXXV – XXXVIII) which is a electron transfer reaction by hydrazine – hydrate (Raney Nickel catalysed) reduction occurred at room temperature with better yields confirmed that hydrazine hydrate – Raney Nickel is better reducing agent ($Ni \rightarrow \frac{1}{2} Ni^{++} + 0.263\ V + N_2 H_5 + 3H+$) than zinc – acetic acid combination ($Zn \rightarrow \frac{1}{2} Zn^{++} + 0.761$). Reduction of vinylnitro steroids (XXXI – XXXIV) afforded steroidal ketones (XXXV – XXXVIII) we have used these ketones (XXXV – XXVIII) for 2D – NMR spectral studies.

^{1}H-^{1}H-NMR homonuclear cosy spectrum of 5α-cholestan-6-one (XXXV) (Fig. 1a) :

^{1}H-^{1}H-NMR homonuclear cosy spectrum of 5α-cholestan-6-one (XXX) (Fig. 1a) explains that H-5α at (δ 2.18) as double doublet (Jae = 4.5 Hz, Jaa = 13.5 Hz, axial) was coupled with H-4α (δ 2.04) and H-4β (δ 2.00). H-7β (δ 2.16) appeared as double doublet (Jae = 4.5 Hz and Jgem=13.0 Hz) was coupled with H-7α (δ 2.14) and H-8β (δ 1.86) and H-7α (δ 2.14) was coupled with H-7β (δ 2.16) and H-8β (δ 1.86).

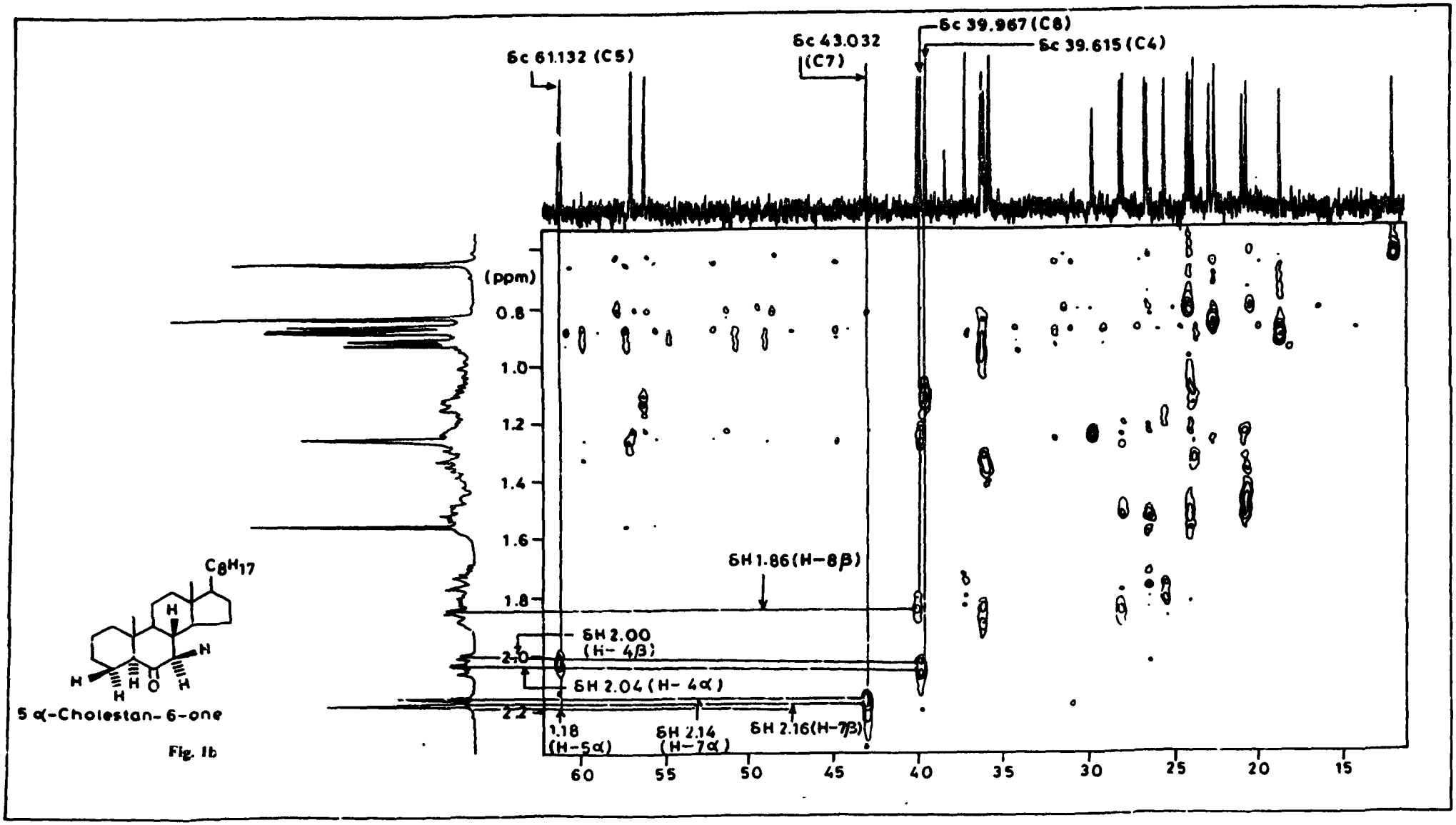
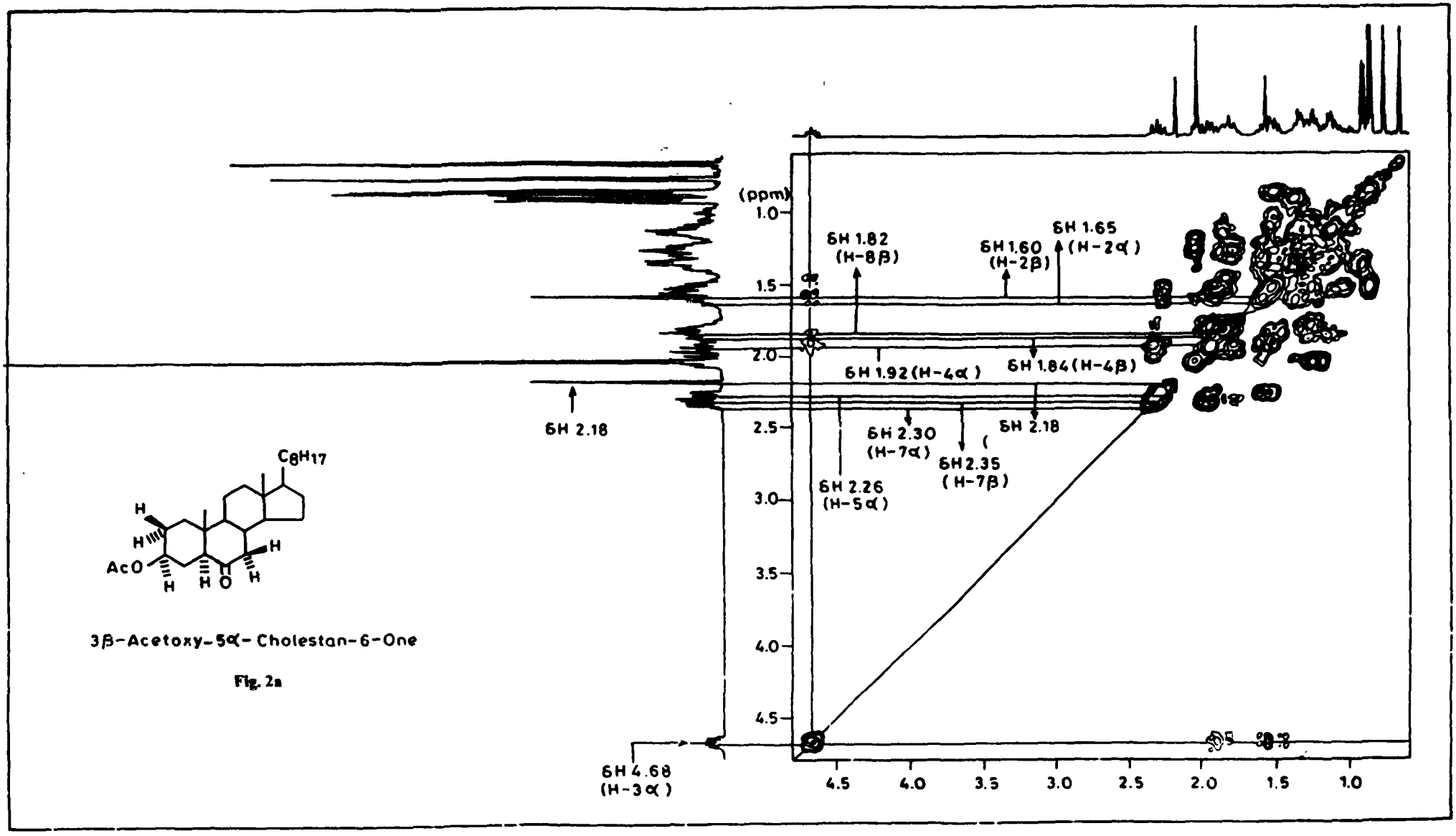

δc 61.132 (C5)
δc 43.032 (C7)
δc 39.967 (C8)
δc 39.615 (C4)
(ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
δH 1.86 (H-8β)
δH 2.00 (H-4β)
δH 2.04 (H-4α)
1.18 (H-5α)
δH 2.14 (H-7α)
δH 2.16 (H-7β)
60
55
50
45
40
35
30
25
20
15
C8H17
H
H
H
H
O
H
5 α-Cholestan-6-one

Fig. 1b



3β-Acetoxy-5α-Cholestan-6-One

Fig. 2a

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 5α-cholestan-6-one (XXXV) (Fig. 1b) :

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 5α-cholestan-6-one (XXXV)(Fig. 1b) correlates H-4α (δ 2.04), H-4β (δ 2.00) to δ_C 26.656 (C4), H-5α (δ 2.18) to δ_C 61.132 (C5), H-7β (δ 2.16), H-7α (δ 2.15) to δ_C 43.032 and H-8β (δ 1.86) to 39.976 (C8) (one bond correlation).

^{1}H-^{1}H-NMR homonuclear cosy spectrum of 3β-acetoxy-5α-cholestan-6-one (XXXVI) (Fig. 2a) :

The ^{1}H-^{1}H-NMR cosy spectrum of 3β-acetoxy-5α-cholestan-6-one (XXXVI) (Fig. 2a) gave contour on diagonal at δ 4.68 (H - 3α) which is coupled by H - 4α (δ 1.92), H - 4β (δ 1.84), H - 2α (δ 1.65) and H-2β (δ 1.60). H - 5α (δ 2.26) appeared as double doublet (Jae = 4.5 Hz, Jaa = 12 Hz) coupled by H - 4α (δ 1.92) and H - 4β (δ 1.84). A singlet at δ 2.18 in the ^{1}H – ^{1}H cosy spectrum is assigned to protons of acetate methyl. The contour at δ 2.18 on diagonal has no cross over multiplet and therefore acetate methyl protons (δ 2.18) appeared as signalet. The H - 7β appeared as double doublet at δ 2.35 (J = 4.5 Hz and 12 Hz). This proton is coupled by H - 7α (δ 2.30) and H - 8β (δ 1.82).

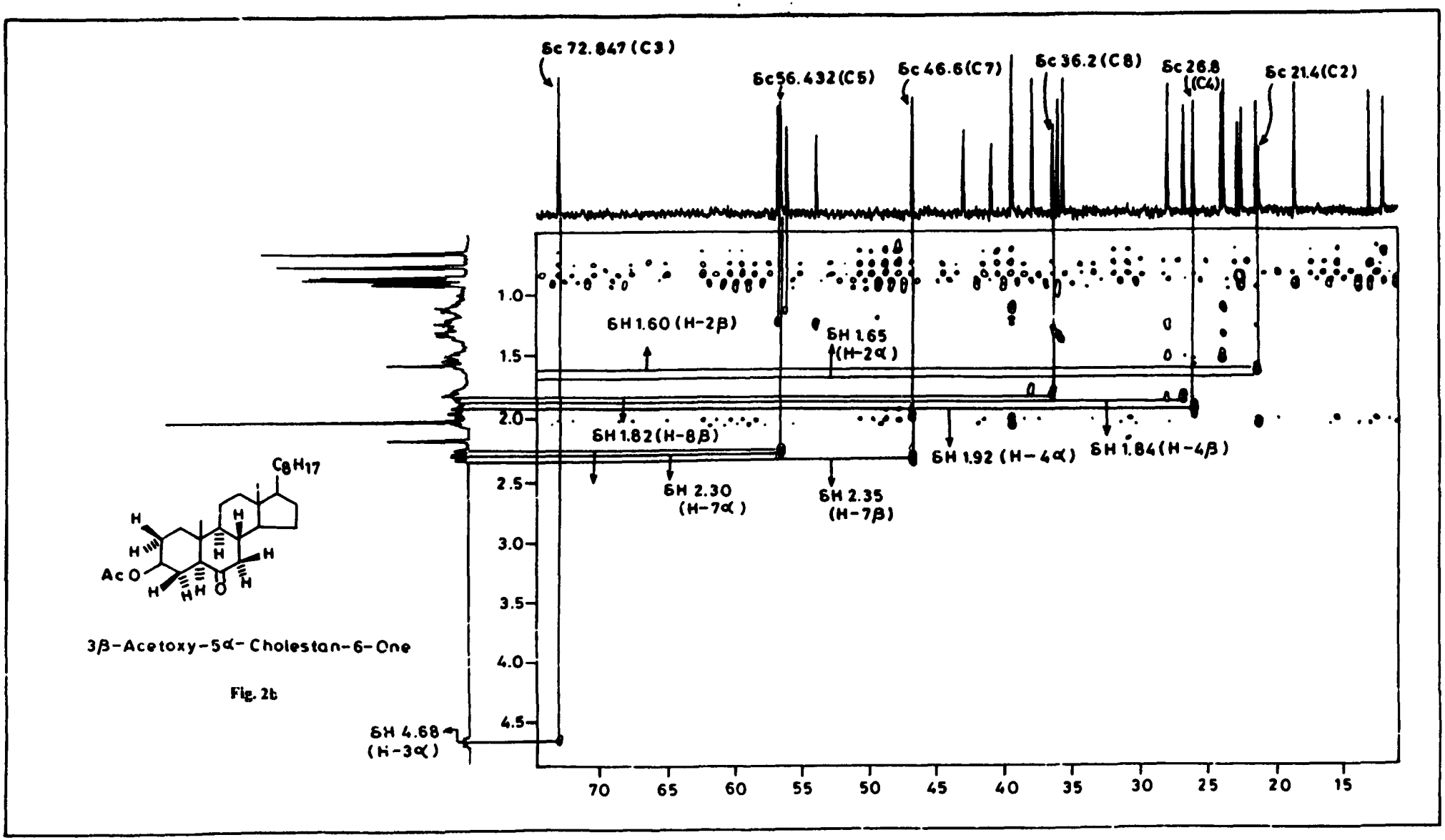
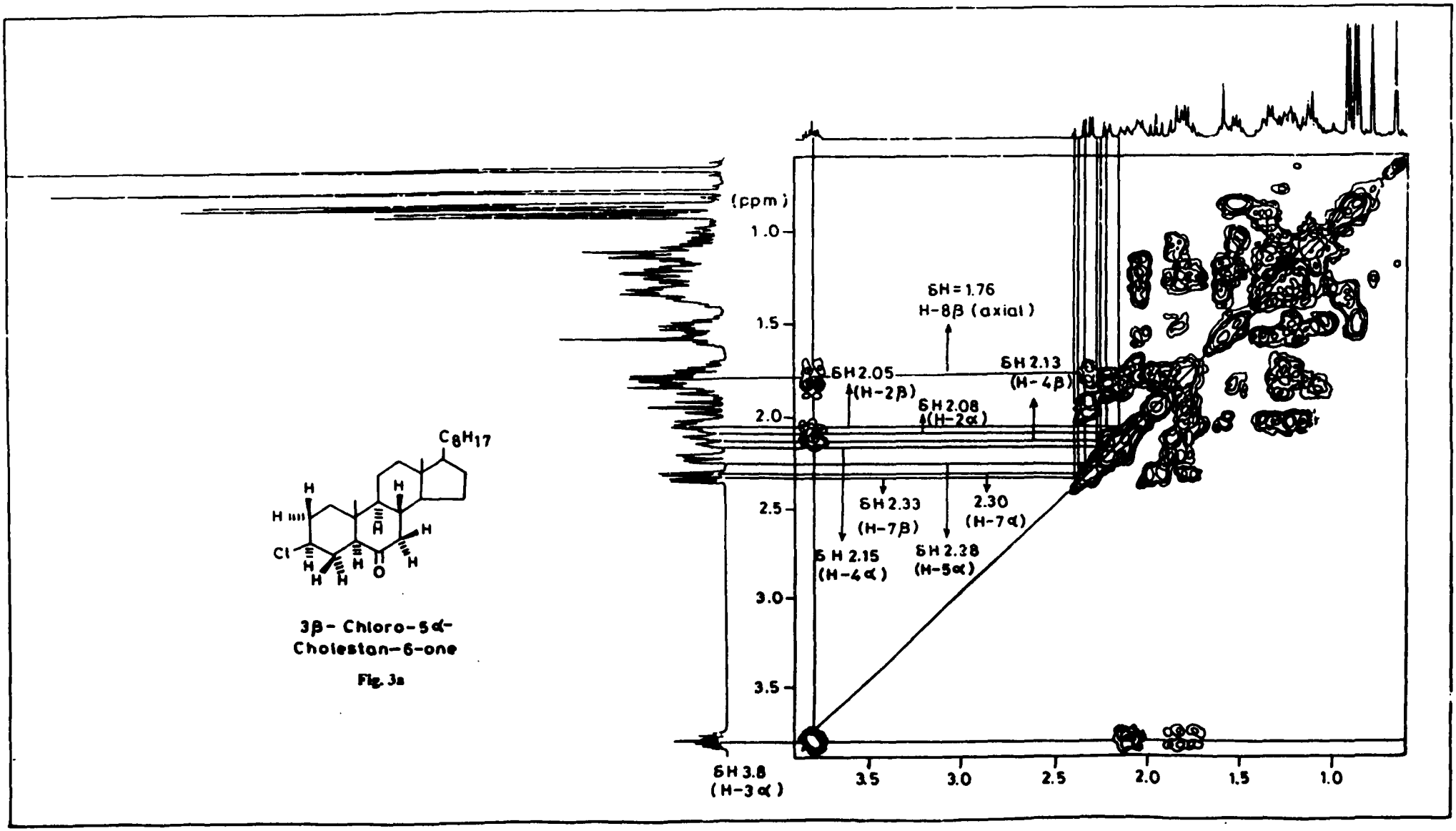

δc 72.847 (C3)
δc 56.432 (C5)
δc 46.6 (C7)
δc 36.2 (C8)
δc 26.8 ((C4)
δc 21.4 (C2)
δH 1.60 (H-2β)
δH 1.65 (H-2α)
δH 1.82 (H-8β)
δH 1.92 (H-4α)
δH 1.84 (H-4β)
δH 2.30 (H-7α)
δH 2.35 (H-7β)
δH 4.68 (H-3α)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
70
65
60
55
50
45
40
35
30
25
20
15
C8H17
AcO
3β-Acetoxy-5α-Cholestan-6-One

Fig. 2b



3β- Chloro-5α-
Cholestan-6-one

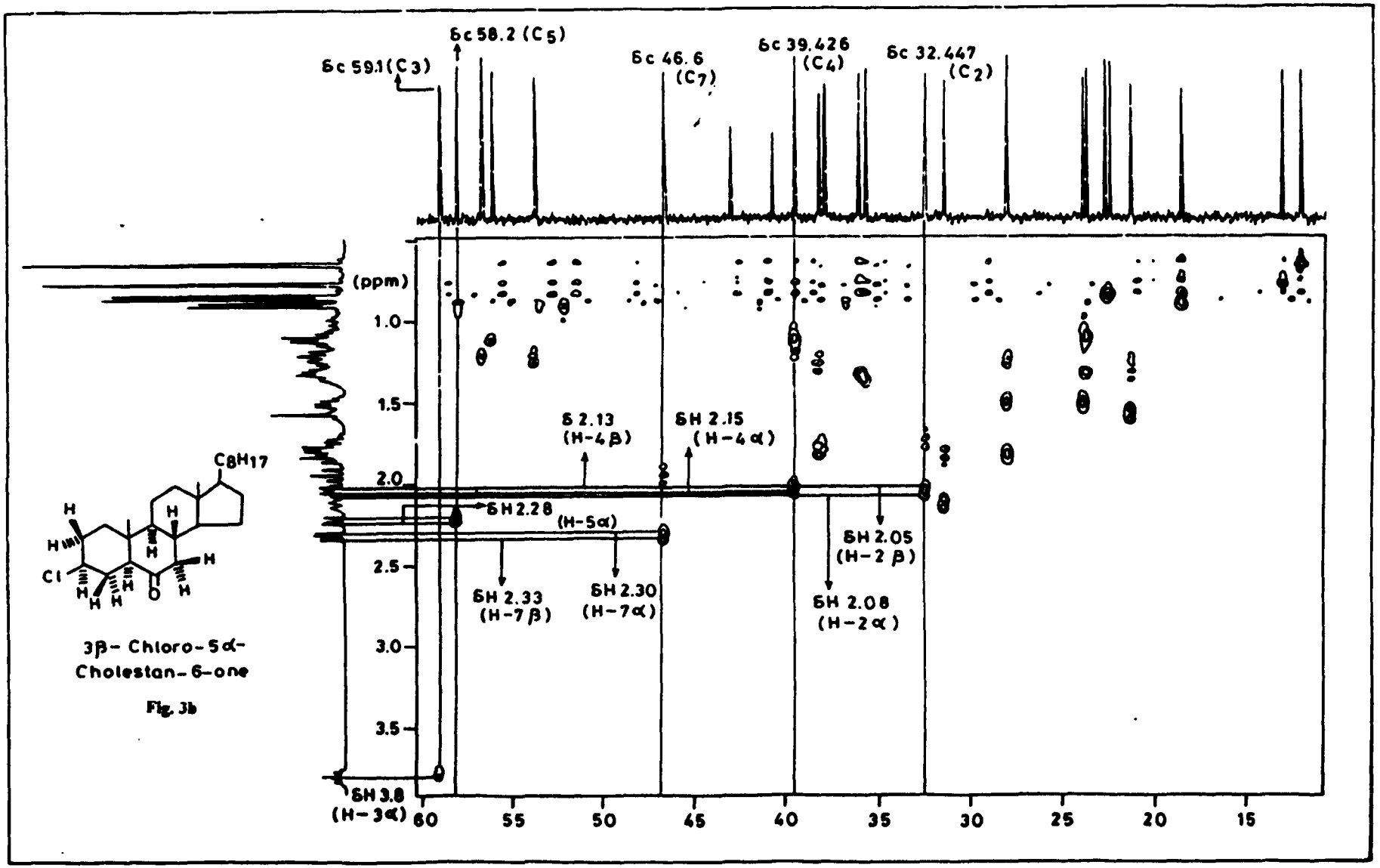
Fig. 3a

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β- acetoxy -5α-cholestan-6-one (XXXVI) (Fig. 2b) :

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β-acetoxy-5α-cholestan-6-one (XXXVI) (Fig. 2b) correlates the δ (chemical shift) of protons with their ^{13}C value. δ 4.68 (H - 3α) is correlated to δ 72.847 (C3), δ 2.26 (H-5α) to δ_C 56.432 (C5), δ_C 2.35 (H - 7β) and δ 2.30 (H - 7α) to δ_C 46.6 (C7).

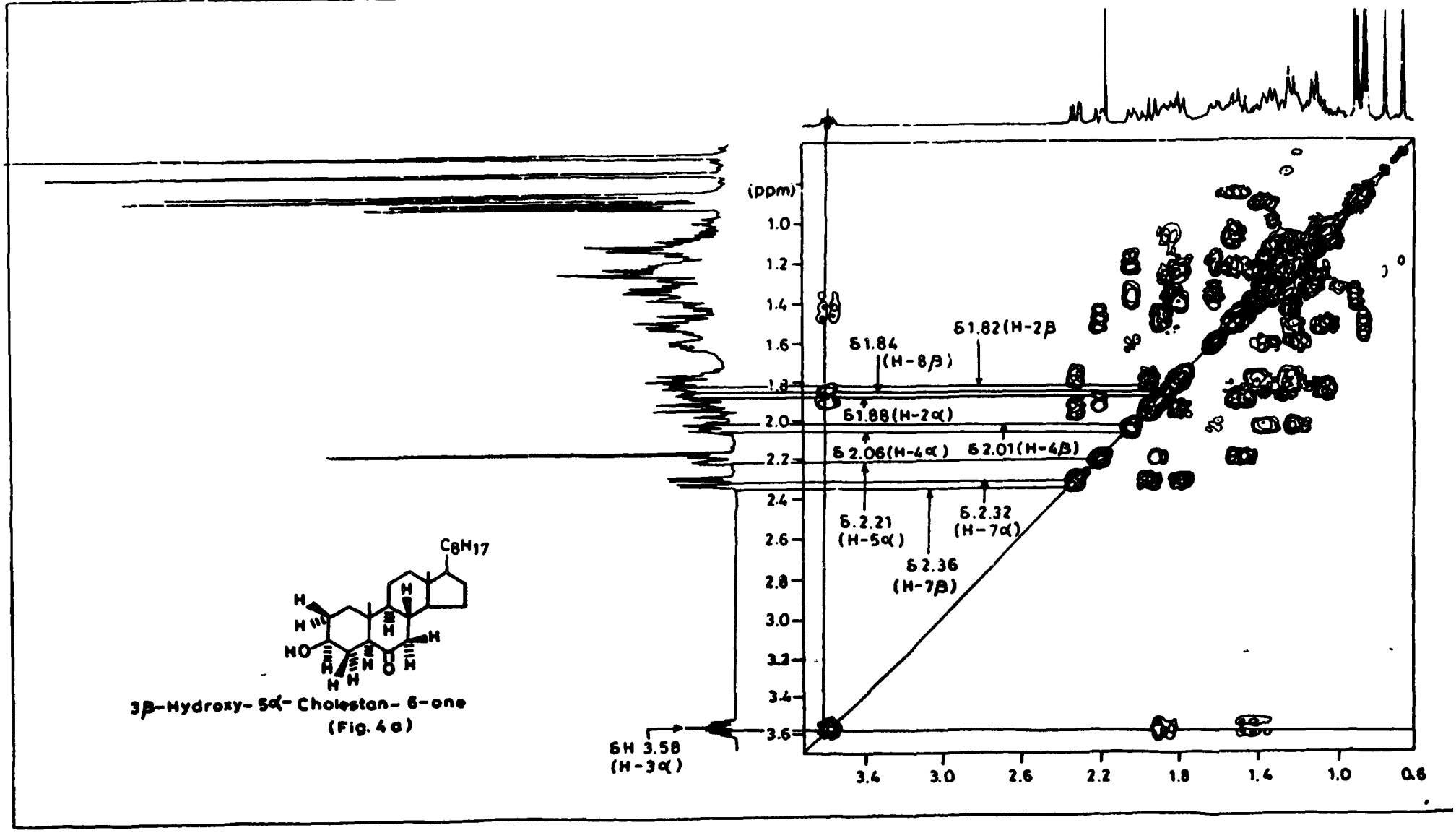
^{1}H–^{1}H–NMR homonuclear cosy spectrum of 3β-chloro-5α-cholestan-6-one (XXXVII) (Fig. 3a) :

^{1}H–^{1}H–NMR homonuclear cosy spectrum of 3β-chloro-5α=-cholestan-6-one (XXXVII) (fig. 3a) gave a multiplet at δ 3.8 (H - 3α) as contour on the diagonal and known as diagonal peak multiplet and on either sides of diagonal cross peak multiplets at δ 2.13 (H - 4β), δ 2.15 (H - 4α), δ 2.05 (H - 2β) and δ 2.08 (H - 2α) coupling with H - 3α which appeared as multiplet. A double doublet at δ 2.33 (Jae = 4.5 Hz and Jgem = 13 Hz) was assigned to H - 7β. This proton being equatorial is coupled by H - 8β (δ 1.76, axial) and H - 7α (δ 2.30, axial) which is correlated by ^{1}H–^{1}H–NMR cosy spectrum. A double



3β- Chloro-5α-Cholestan-6-one

Fig. 3b



3β-Hydroxy-5α-Cholestan-6-one
(Fig. 4a)

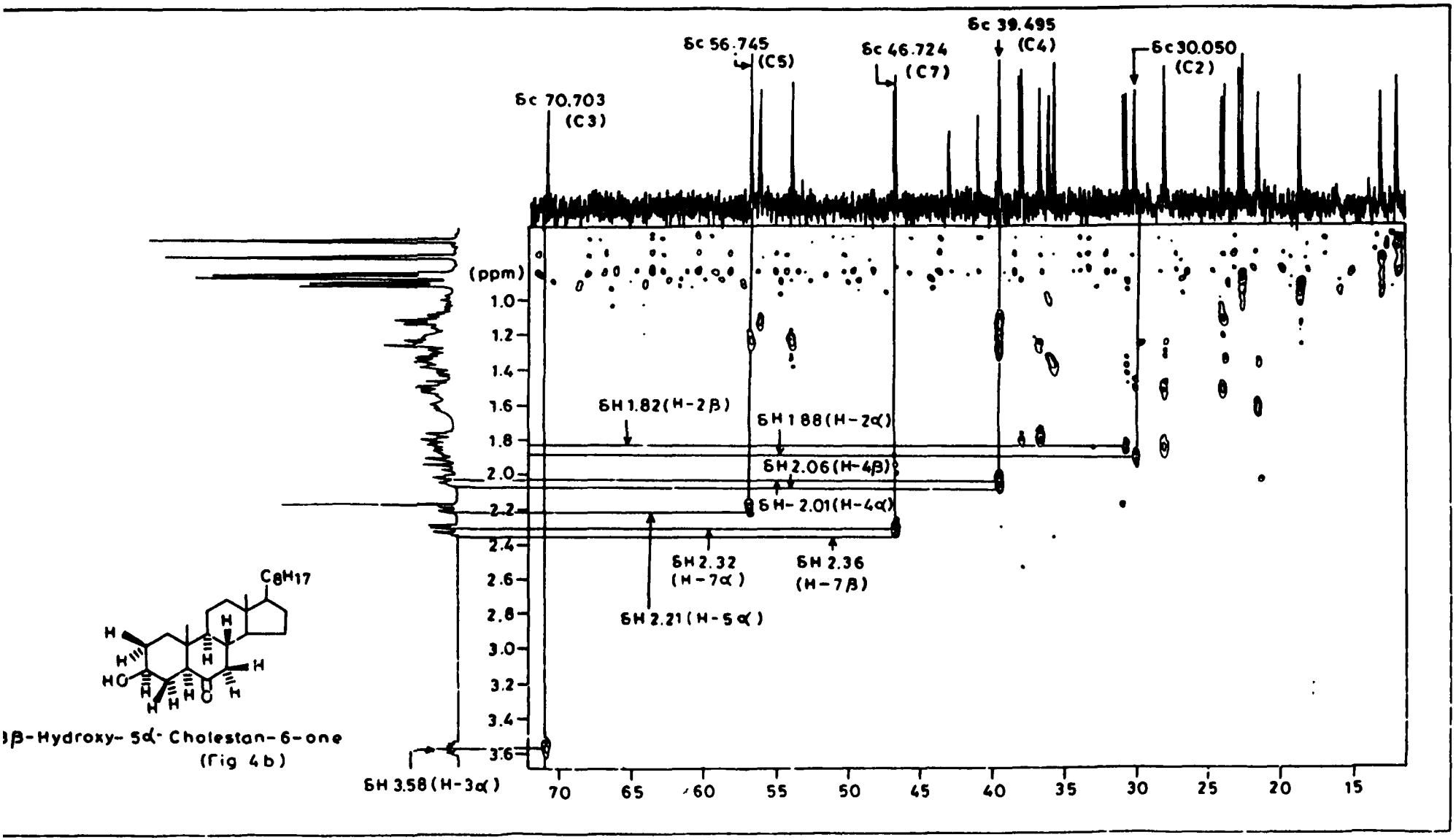
doublet at δ 2.28 (Jae = 4.5 Hz and Jaa = 13 Hz) for one proton is assigned to H - 5α. This proton is coupled by H - 4α (δ 2.15) and H - 4β (δ 2.13).

^{1}H–^{13}C–NMR hetronuclear cosy spectrum of 3β-chloro-5α-cholestan-6-one (XXXVII) (Fig. 3b) :

Chemical shifts (δ) are easily correlated with δ_C; δ 3.8 (H - 3α) is correlated with δ_C 59.1 (C3), δ 2.28 (H - 5α) to δ_C 58.2 (C5), δ 2.33 and δ 2.30 to δ_C 46.532 (C7) and δ 2.05 (H - 2β) and δ 2.08 (H - 2α) are finally correlated to δ_C 32.447 (C2).

^{1}H-^{1}H-NMR homonuclear cosy spectrum of 3β-hydroxy-5α-cholestan-6-one (XXXVIII) (Fig. 4a) :

^{1}H-^{1}H-NMR homonuclear cosy spectrum of (XXXVIII) (Fig. 4a) has made it clear that H-3α gives peak at δ 3.58 (W½ = 18 Hz) as multiplet was coupled by H-4α (δ 2.06), H-4β (δ 2.01), H-2α (δ 1.88) and H-2β (δ 1.82). H-5α at (δ 2.21) was coupled by H-4α (δ 2.06) and H-4β (δ 2.01). H-7β (δ 2.36) was coupled by H-7α (δ 2.32) and H-8β (δ 1.84). Coupling was observed between H -7α (δ 2.32) and H-7β (δ 2.36), H-8β (δ 1.84).



3β-Hydroxy-5α-Cholestan-6-one
(Fig 4b)

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β-hydroxy-5α-cholestan-6-one (XXXVIII-b) (Fig. 4b) :

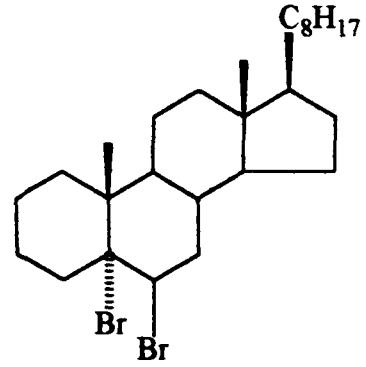
^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β-hydroxy-5α-cholestan-6-one (XXXVIII) (Fig. 4b) has shown that H-2α (δ 1.88), H-2β (δ 1.82) was correlated to δ_C 30.050 (C2), H-3α (δ 3.58) to δ_C 70.703 (C3), H-4α (δ 2.06), H-4β (δ 2.01) to δ_C 39.495 (C4), H-5α (δ 2.21) to δ_C 56.745 (C5), H-7β at (δ 2.36), H-7α (δ 2.32) to δ_C 46.724 (C7). The H-8β was correlated to δ_C 37.909 (C8).

CHAPTER-THREE

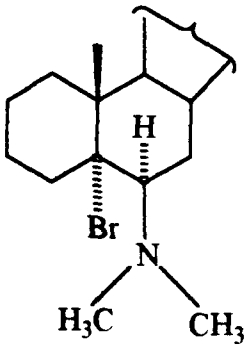
Reactions of Dibromosteroids with Organic Bases.

The manifold physiological properties associated with a variety of compounds containing hetero atoms with useful therapeutic values prompted us to carry out extensive research in this field. Steroids a class of biologically active compounds were modified to a variety of oxygen and nitrogen containing derivatives, playing a vital role in the era of medicine and drugs and synthetic organic chemistry. These compounds were found to possess dermatological, opthalmic, antiulcer, immunoassay and CNS depressant activities in association with other physiological activities. The present work describes the reaction of 5, 6β-dibromo-5α-steroids (XXXIX) and 3 keto-5, 6β-dibromo-5α-steroids (XL) with dimethylamine, succinimide and morpholine. The reactions of 5, 6β-dibromo-5α-cholestane (XXXIX) in benzene with dimethylamine, succinimide and morpholine at room temperature for half on hour, afforded 5-bromo-6β-dimethylamino-5α-choleatne (XLI), 5-bromo-6β-amino-5α-cholestane (XLII) and 5 bromo-6β-morpholino-5α-cholestane (XLIII) respectively. When 5, 6β-dibromo-5α-cholestan-3-one (XL) was treated with morpholine and 2-aminopyrimidine under identical reaction conditions gave 6β-morpholinocholest-4-en-3-one (XLIV) and 6β-aminopyrimidinocholest-4-en-3-one (XLV). The structure of

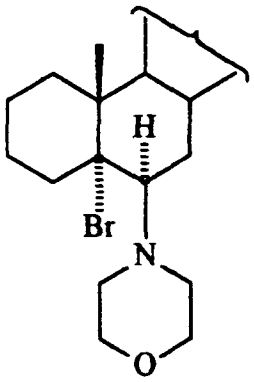
these compounds was established on the basis of analytical and spectral (IR, ^{1}H-NMR, ^{13}C-NMR and Mass) evidences.



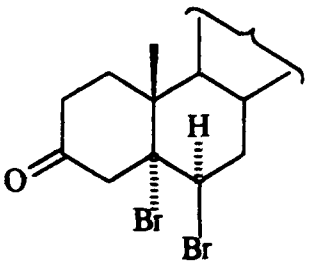
(XXXIX)



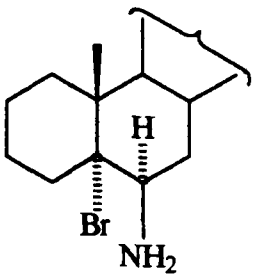
(XLI)



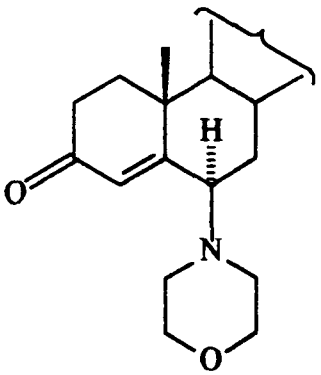
(XLIII)



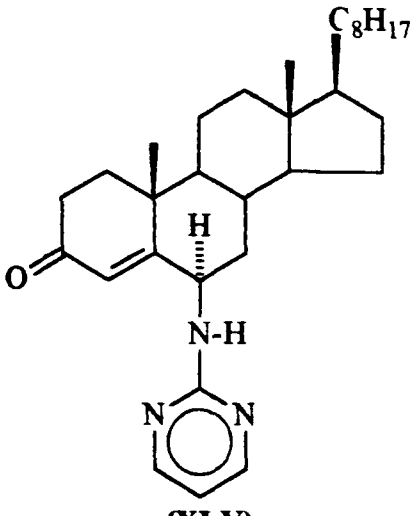
(XL)



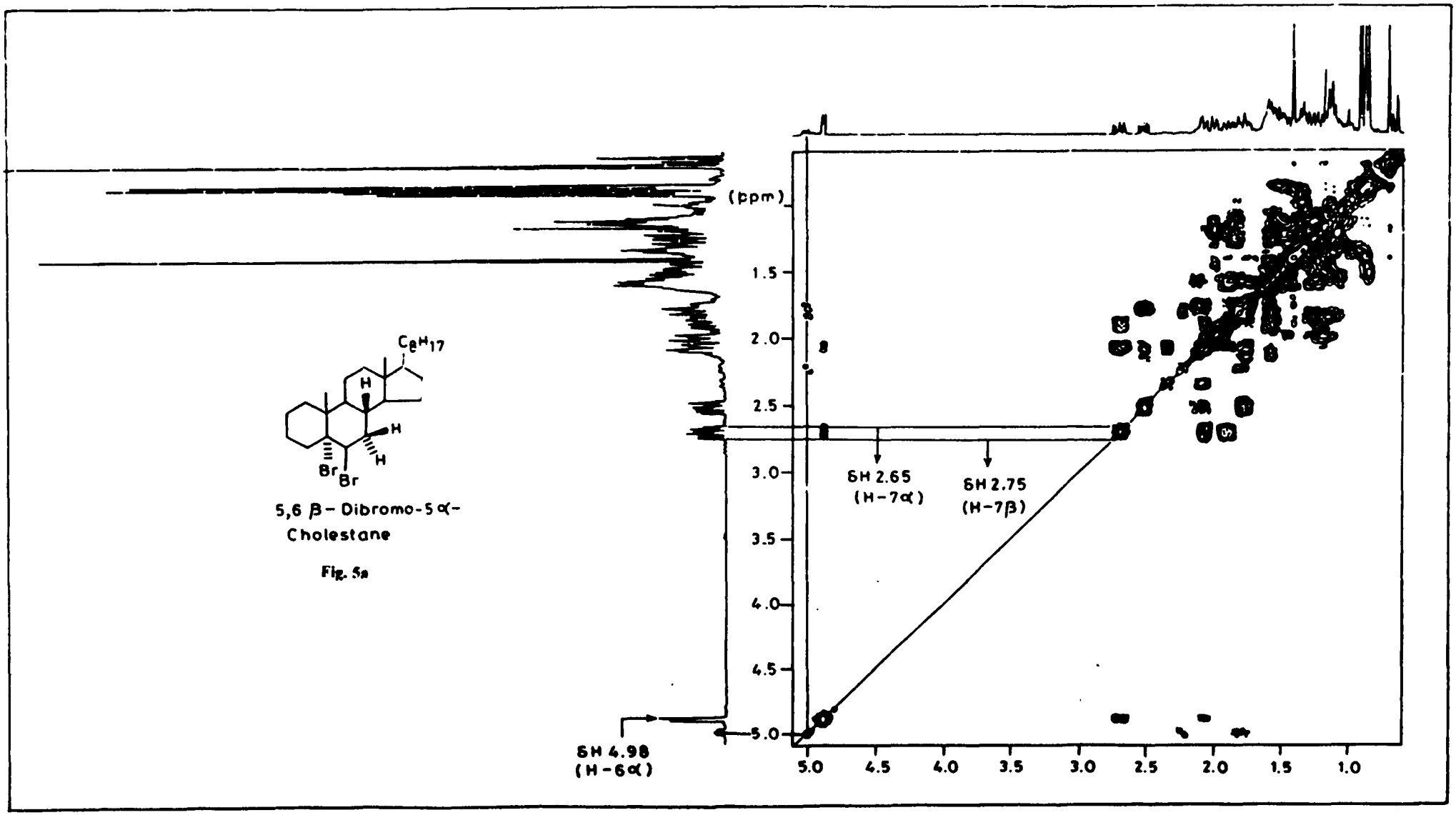
(XLII)



(XLIV)

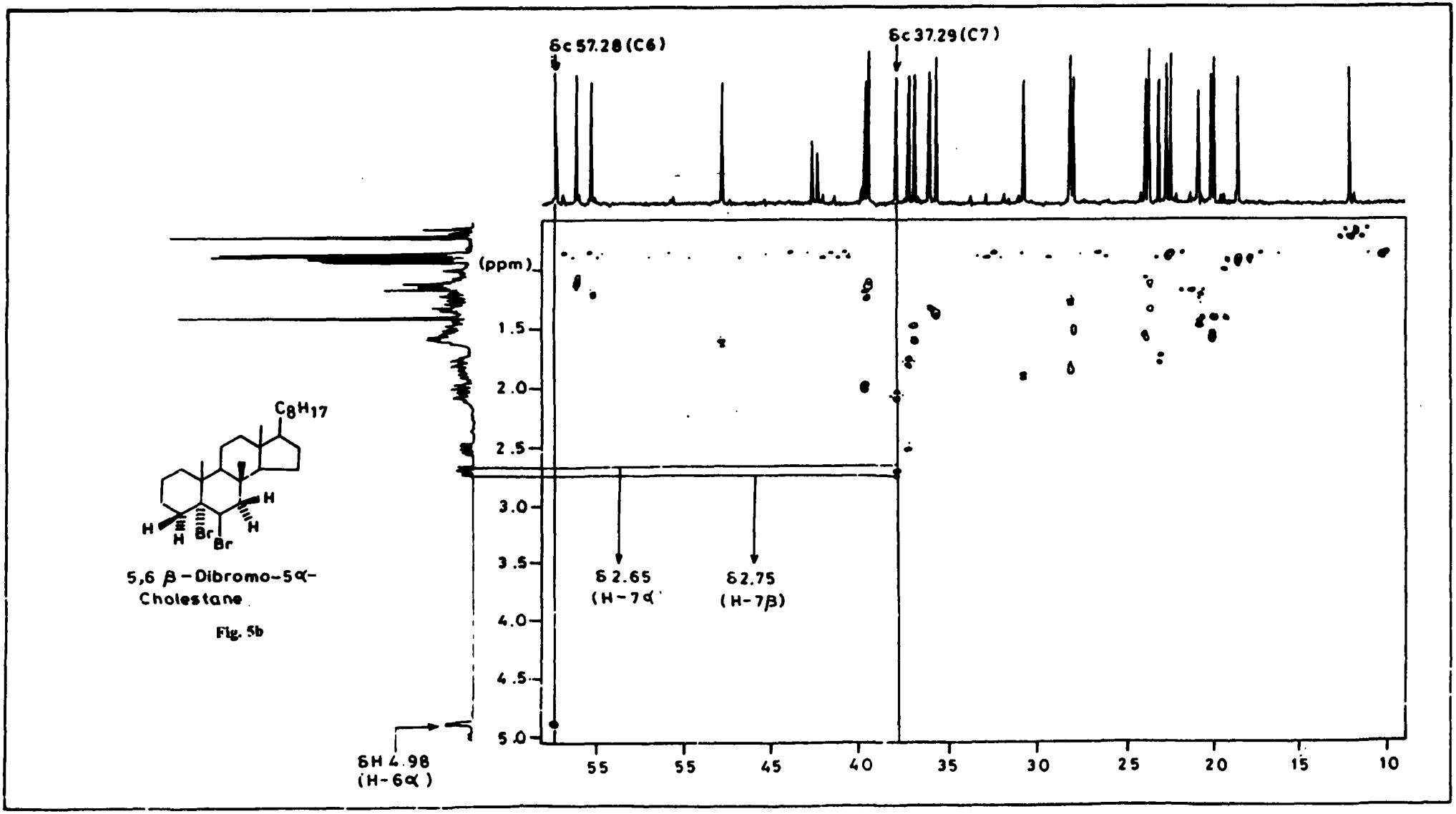


(XLV)



5,6 β – Dibromo-5α-
Cholestane

Fig. 5a

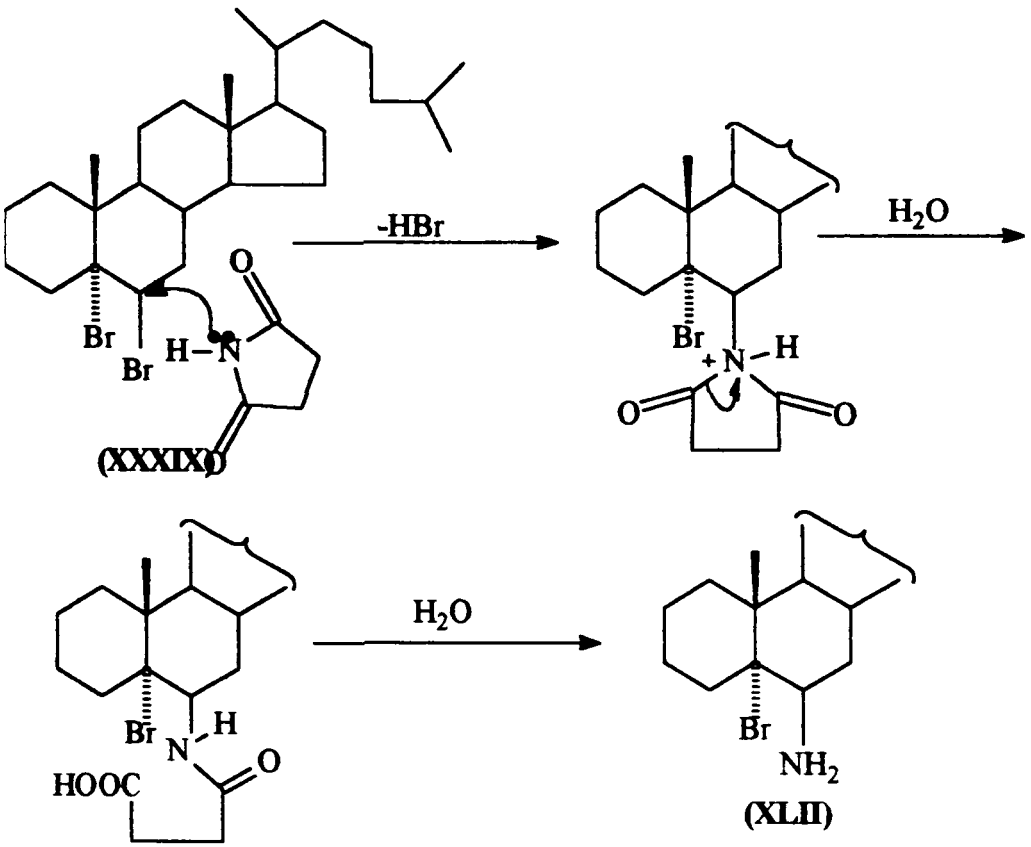


5,6 β-Dibromo-5α-Cholestane

Fig. 5b

The spectral studies of 5, 6β-dibromo-5α-cholestane (XXXIX) the starting compound was done in detail with special reference to 2D-NMR spectroscopy to support the orientation of bromine atoms at C5 (α-oriented, axial) and C6 (β-oriented, axial) and its purity. ^{1}H-^{1}H-NMR homonuclear and ^{13}C-NMR hetronuclear cosy spectrum (Fig. 5a,b) correlated H-6α (δ 4.98) which appeared as double doublet coupled by H-7α (Jea = 4 Hz) and H-7β (Jee = 2 Hz) to δ_C57.28 and H-7α (δ 2.65) and δ H-7β (δ 2.75) to δ_C 37.29 (C7).

Formation of products (XLI, XLIII, XLIV and XLV) involved simple substitution reaction where bromine is replaced by dimethylamine, morpholine and 2-aminopyrimidine, but the formation of 5-bromo-6β-amino-5α-cholestane (XLII) obtained by the reaction of 5, 6β-dibromo-5α-cholestane (XXXIX) with succinimide in benzene can be explained by following mechanism

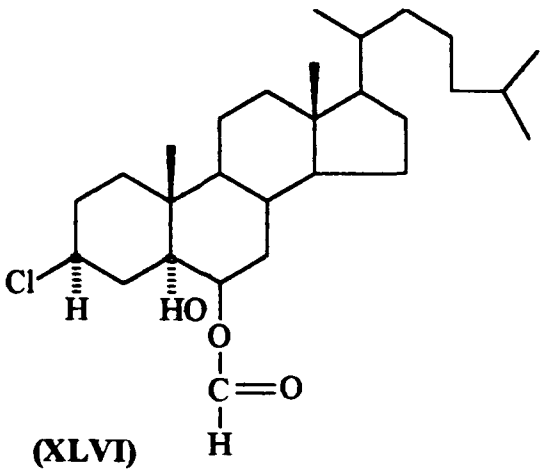

-HBr
H_2O
Br
Br
H–N
O
(XXXIX)
Br
+N–H
O
O
H_2O
Br
N
H
HOOC
O
Br
NH_2
(XLII)

CHAPTER-FOUR

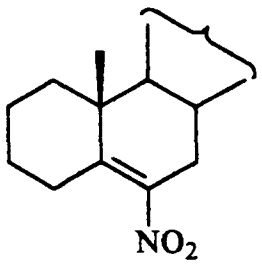
Applications of X-rays In Structure Elucidation of Steroids :

In recent years X-rays methods have been increasingly used qualitative and quantitative analysis as well as for fundamental studies of the properties and structure of various class of both organic and inorganic compounds. X-ray diffraction is the only convenient and hence widely used physical procedure for the complete determination of molecular structure.

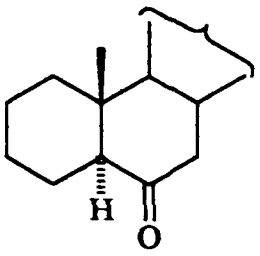
Previous work from these laboratories described crystallographic studies of 3β-chloro-6β-formyloxy-5α-cholestan-5-ol (XLVI), 6-nitrocholest-5-ene (XLVII), 5α-cholestan-6-one (XLVIII) and 3β-chloro-6-nitrocholest-5-ene (XLIX).



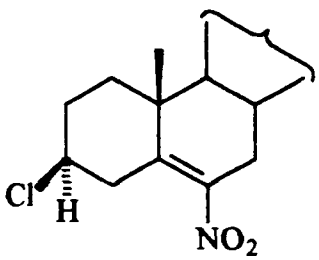
(XLVI)



(XLVII)

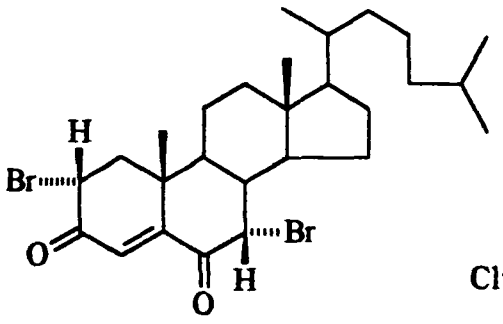


(XLVIII)

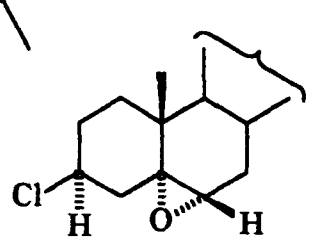


(XLIX)

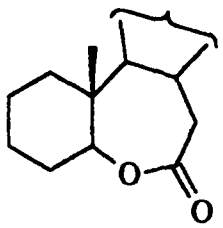
In continuation, as part of X – ray studies of steroids we have taken few steroidal compounds of cholestane series synthesized in our laboratory such as 2α, 7α-dibromocholest-4-ene-3, 6-dione (L), 3β-chloro-5, 6α-epoxy-5α-cholestane (LI), 6-oxa-B-homo-5α-cholestan-7-one (LII) and 5α-cholestane-3, 6-dione (LIII). In each case different parameters and well as bond length bond angle of various bonds present in each molecule, obtained by X- ray analysis are given. Conformational changes occurred during the formation of these compounds (L – LIII) are also studied.



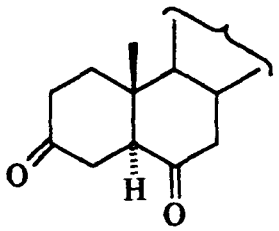
(L)



(LI)



(LII)



(LIII)

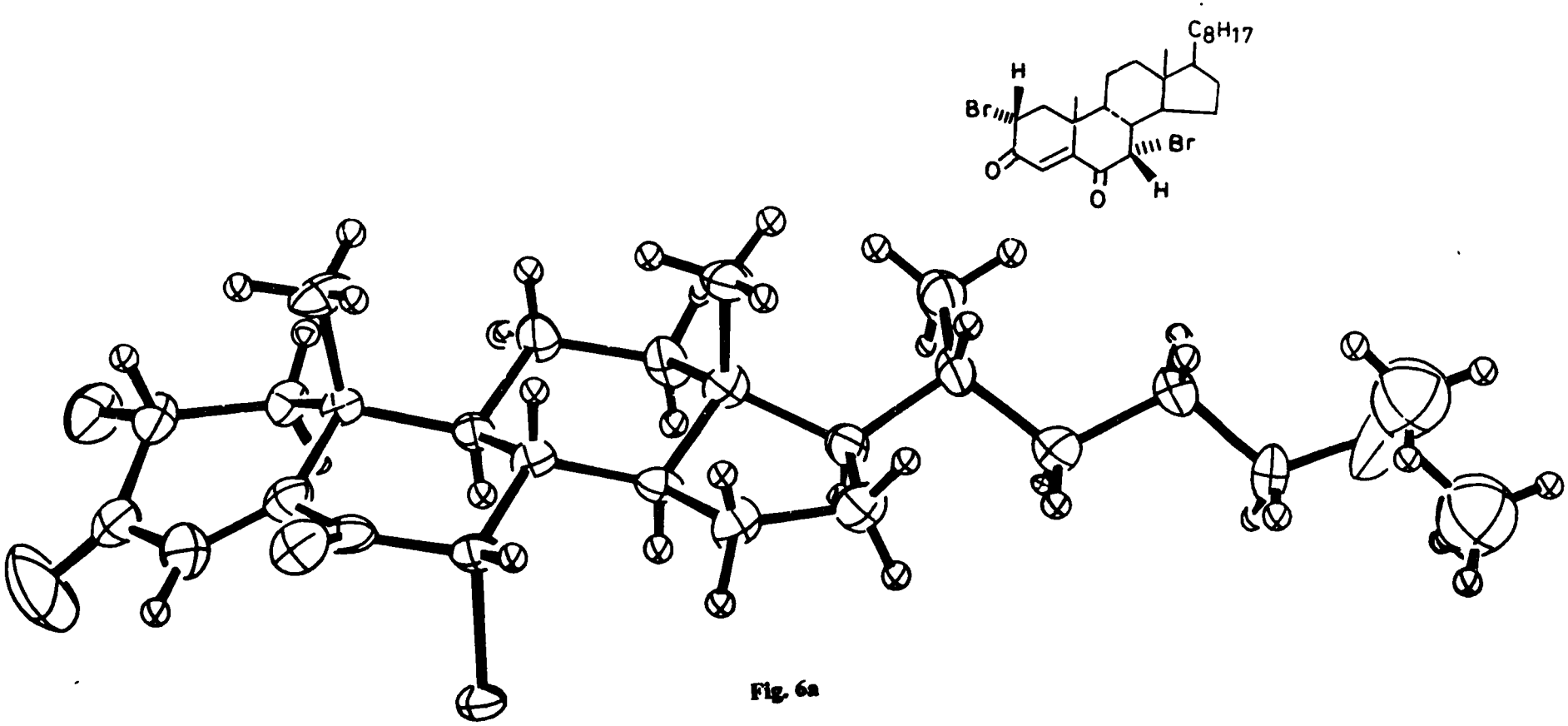


Fig. 6a

General view of the molecule

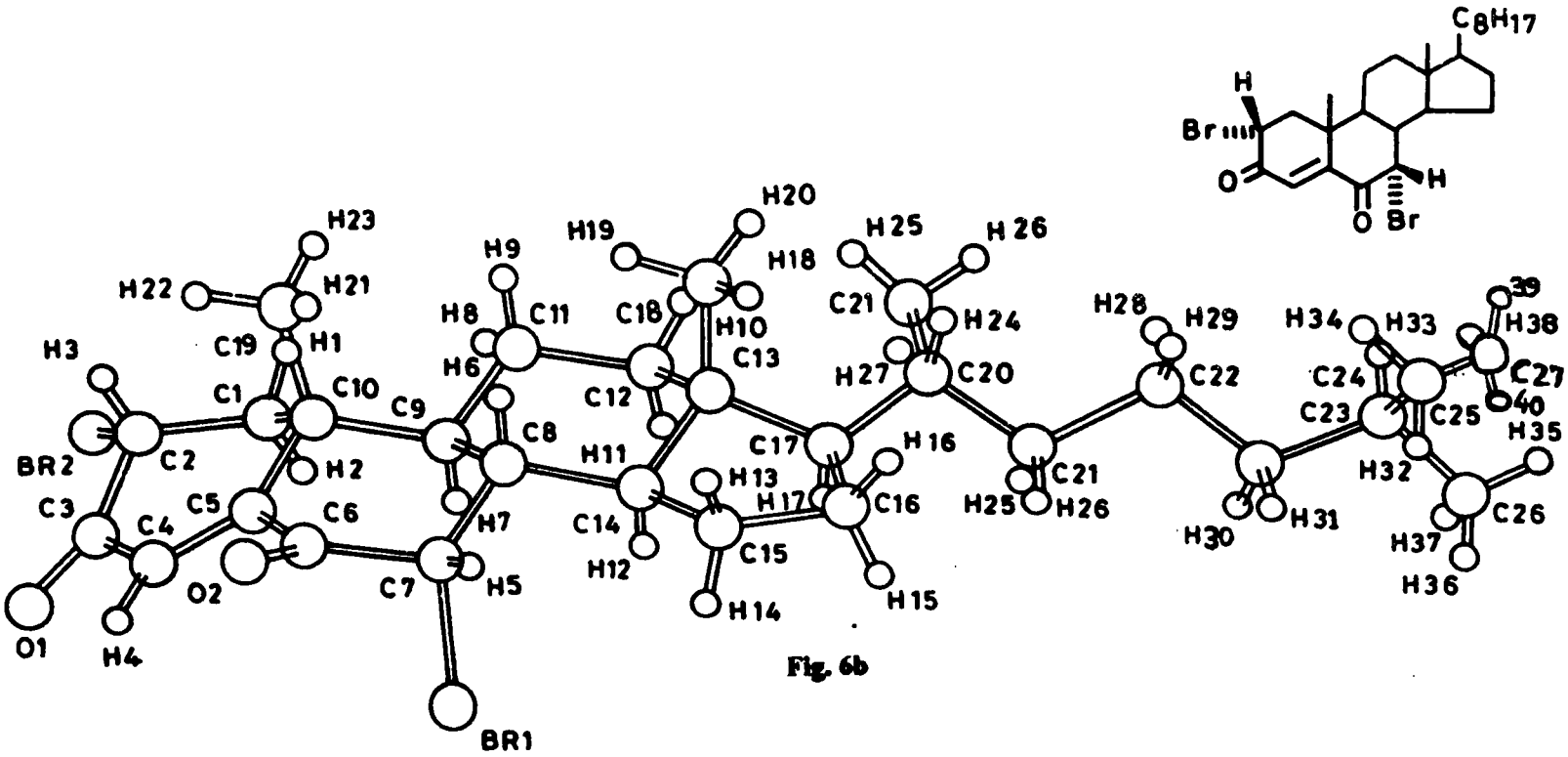


Fig. 6b

Plot showing atomic arrangement and numbering

X-Ray analysis of 2α, 7α-dibromocholest-4-ene-3, 6-dione (L) (Fig. 6a, b) :

The X-ray analysis of 2α, 7α-dibromo cholest-4-ene-3, 6-dione (L) ($C_{27}H_{40}O_2Br_2$, molecular weight = 556.62) was done with crystal morphology results: colourless, prism, crystal dimensions (mm) 0.50 x 0.50 x 0.50, crystal system; monoclinic, lattice dimensions; a = 11.585 (2), b = 7.648 (2), c = 15.323 (1) A°, β = 93.803 (9) A°; volume, 1354.6 (4) $A°^3$, space group, $P2_1$; Z = 2 (two molecules per unit cell), density; 1.364 g/cm^3; radiation, Cukα λ = 1.54178 A°; temperature, 23 °C, structure solutions; direct methods.

In compound 2α, 7α-dibromocholest-4-ene-3, 6-dione (L) (Fig. 6a, b) C2 to C7 carbon atoms with tendency to be in same plane due to Sp^2-nature of the carbons involved causing significant change in the conformation of rings A and B. It is interesting to note that bromine attached to C2 (being equatorial) is lying very close to the plane of the carbonyl group while bromine at C7 (being axial) is moving away from carbonyl plane.

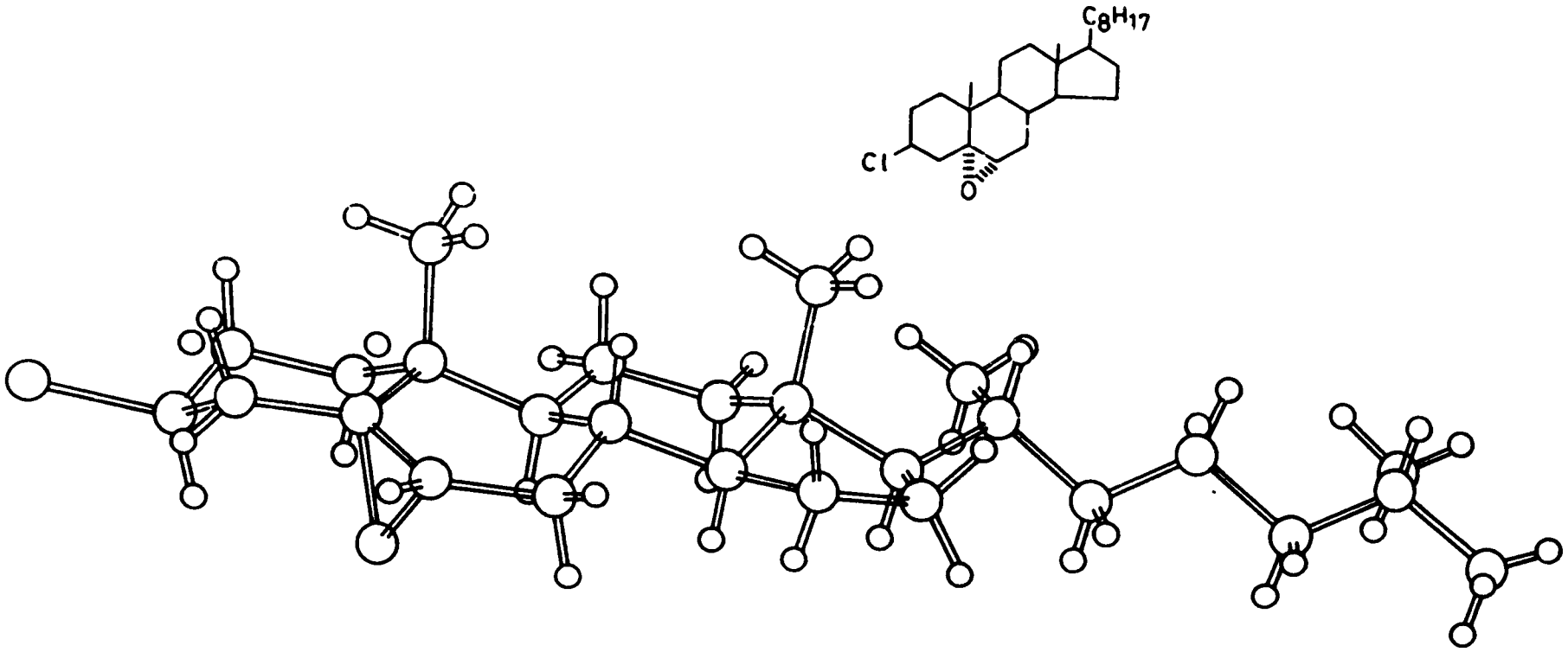


Fig. 7a

General view of the molecule

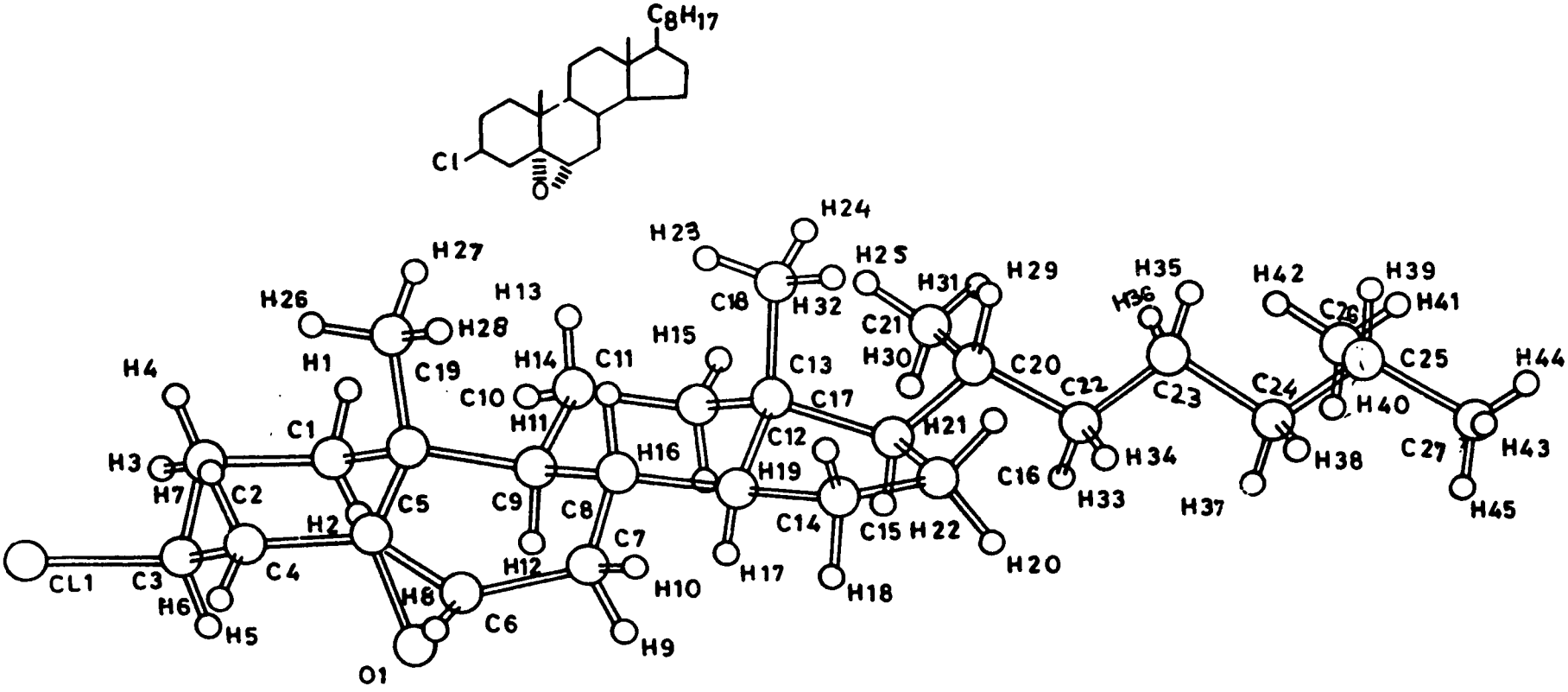


Fig. 7b

Plot showing atomic arrangement and numbering

X-Ray analysis of 3β-chloro-5, 6α-epoxy-5α-cholestane (LI) (Fig. 7a, b) :

X-Ray analysis of 3β-chloro-5, 6α-epoxy-5α-cholestane (LI) was done with the aim to know the conformational changes occurring in the molecule during the formation of epoxide from 3β-chlorocholest-5-ene on treatment with m-chloroperbenzoic acid. It has been found that the conformational changes in rind A and B particularly due to the formation of epoxide ring has occurred. The various parameters obtained during the X-ray crystallographic study : molecular formula; $C_{27}H_{45}ClO$, molecular weight; 421.10, crystal morphology; colourless, plate, crystal dimension (mm); 0.20 x 0.40 x 0.20, crystal system; orthorhombic, lattice parameters; a = 22.80 (4), b = 7.671 (3) 28.657 (5) A°, y = 5012 (2) $A°^3$, space group; $P2_12_12_1$, Z = 8, density 1.116 g/cm^3, radiation; wkα; 1.54178 A°, temperature; 23°C, structure solution; direct methods.

Due to the formation of oxirane ring bond angles around C5 and C6 in compound 3β-chloro-5, 6α-epoxy-5α-cholestane (LI) (Fig. 7a, b) under go changes which are causing strain and geometrical deformation in rings A and B.

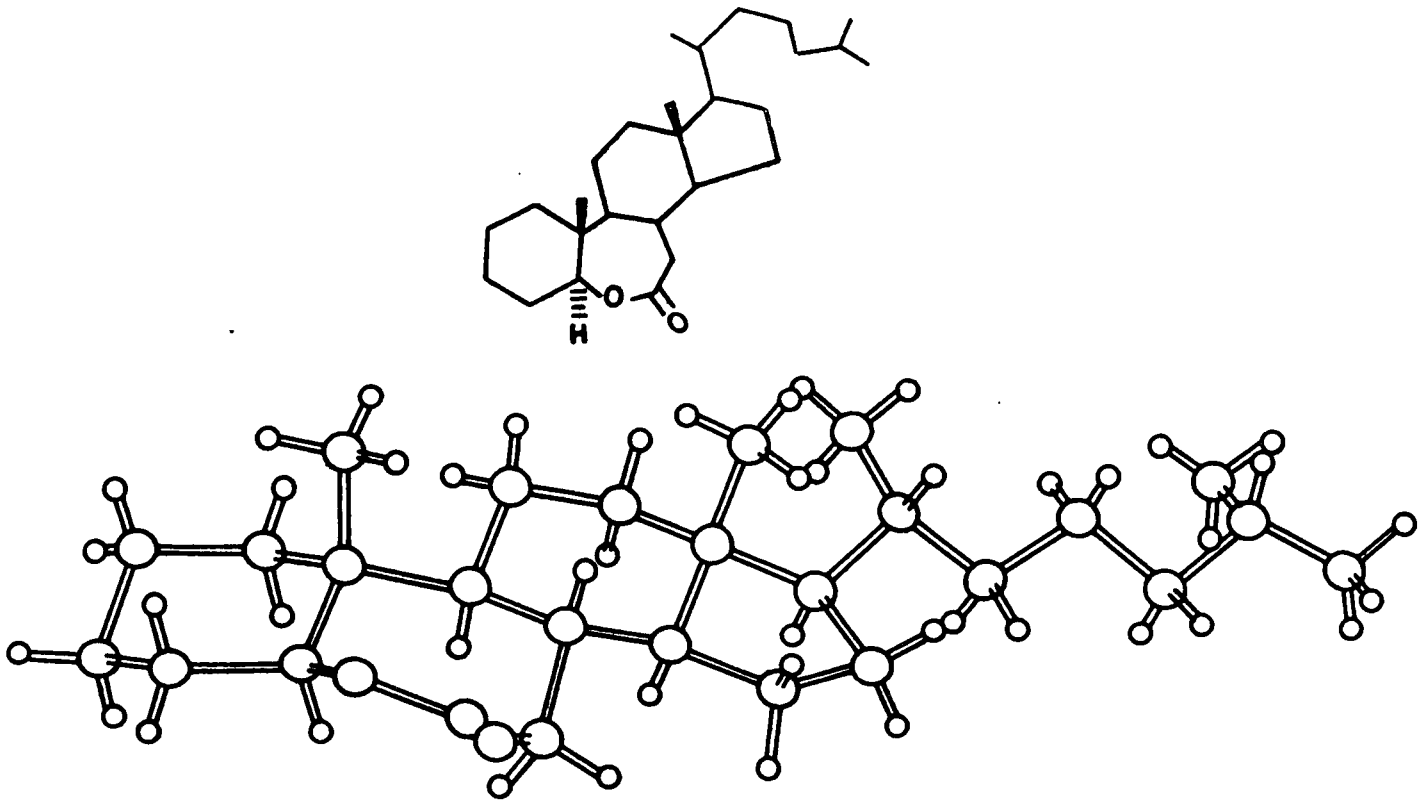


Fig. 8a

General view of the molecule

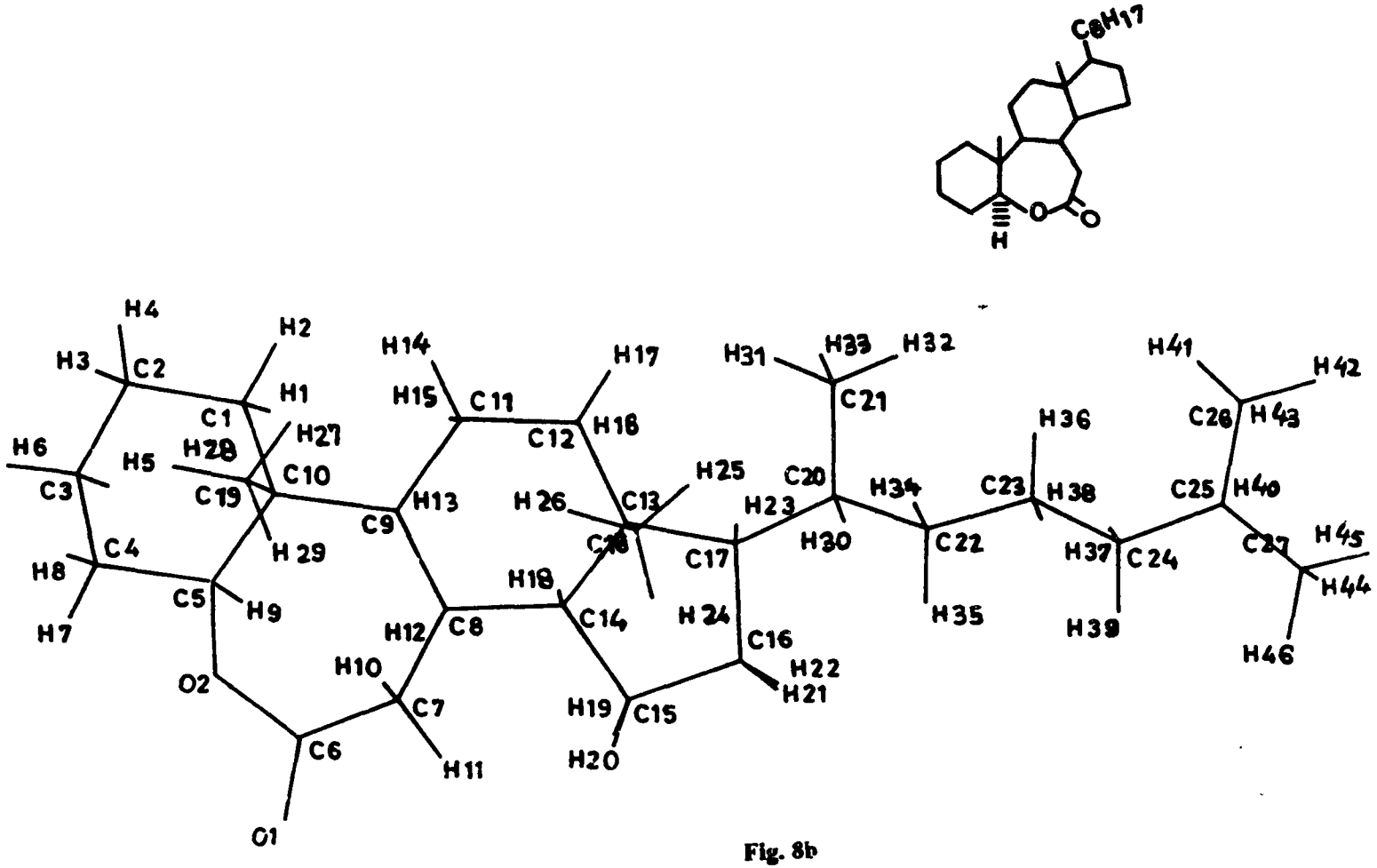


Fig. 8b

Plot showing atomic arrangement and numbering

The X-Ray crystal study of 6-oxa-B-homo-5α-cholestan-7-one (LI) (Fig. 8a, b) :

During the preparation of oxasteroids, when 5α-cholestan-6-one was treated with perbenzoic acid in chloroform (p-toluenesulphonic acid as catalyst) 6-oxa-B-homo-5α-cholestan-7-one (LII) was obtained which was characterized by (IR, ^{1}H-NMR, Mass). The assigned structure was further supported by X-ray crystallography. The result obtained were : molecular formula; $C_{27}H_{46}O_2$, molecular weight; 402.66, crystal morphology; colourless plate; crystal dimension; 0.10x0.20x0.10, crystal system; monoclinic, lattice parameters; a = 5.971 (2), b = 11.043 (1), c = 19.243 (1) A°, space group; $P2_1$, Z = 2 (two molecules per unit cell), density; 1.062 g/cm^3, radiation; Wkα(λ=1.54178 A°), temperature; 23° C structure solution; Paterson method.

Formation of 6-oxa-B-homo-5α-cholestan-7-one (LII) (Fig. 8a, b) from 5α-cholestan-6-one via Baeyer Villiger oxidation involves inlargement of B ring from six to seven membered and lactone moiety having Sp^2-hybridized carbon atom with tendency to have planar geometry causes deformation in seven membered B-ring. It is pertinent to mention that ring C also suffers slight conformational change.

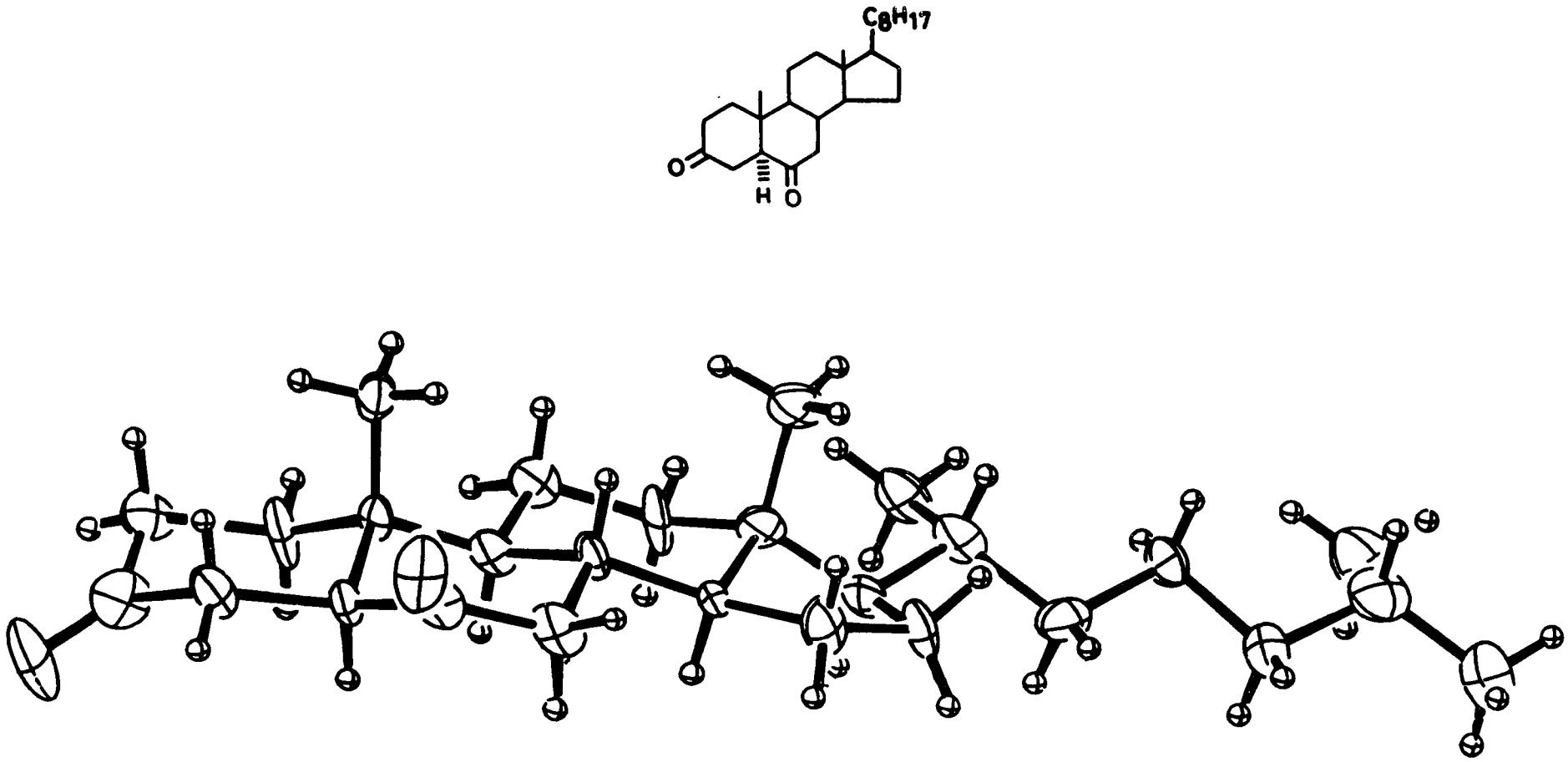


Fig. 9a

General view of the molecule

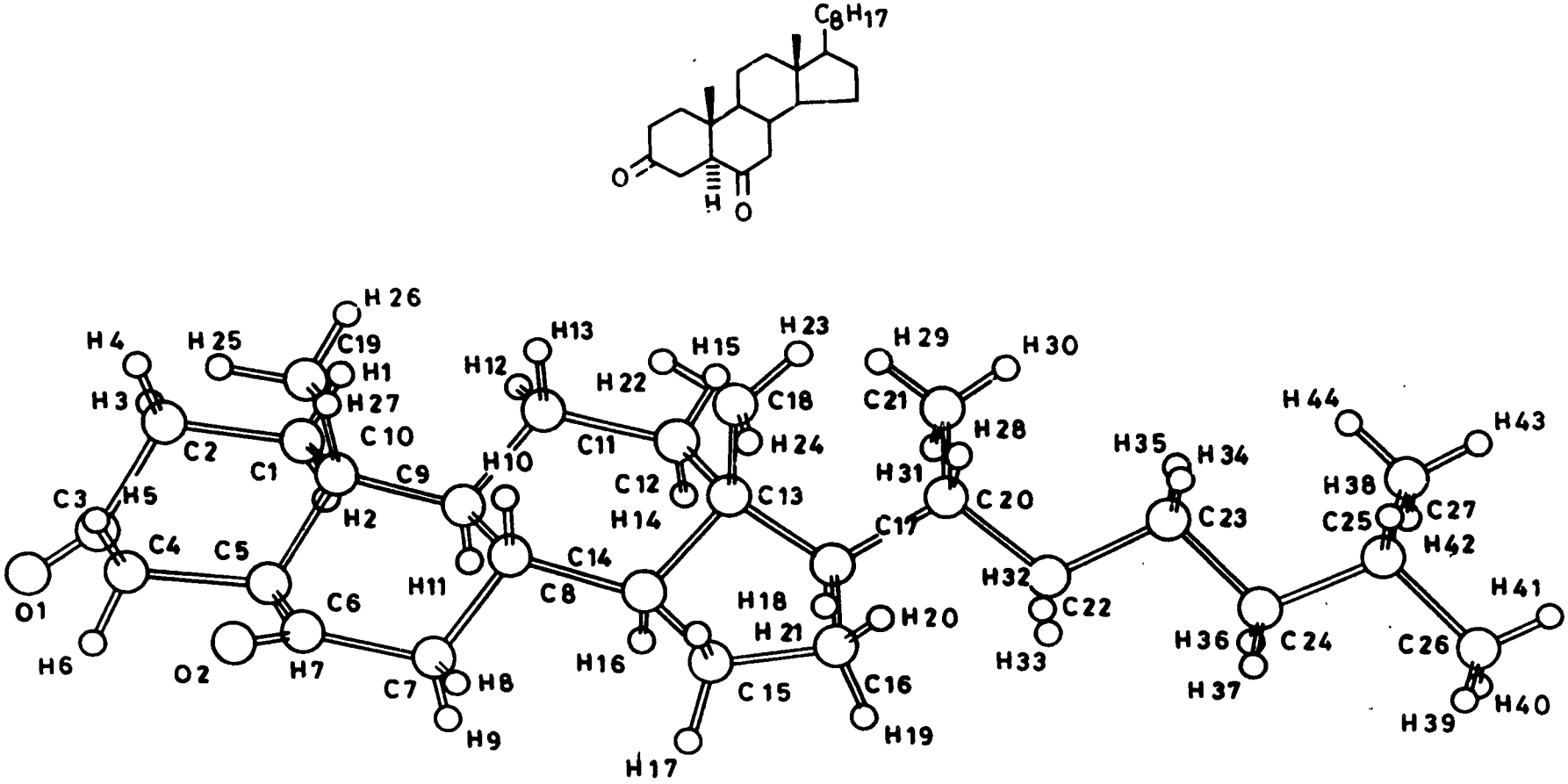


Fig. 9b

Plot showing atomic arrangement and numbering

The X-Ray analysis of 5α-Cholestane-3,6-dione(LIII)(Fig. 9a,b):

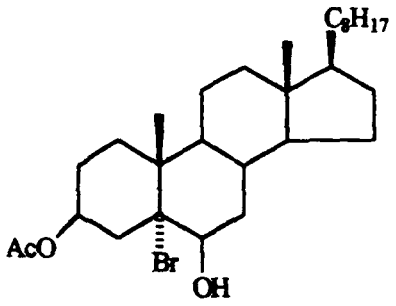
The most interesting compound involved in synthesis of variety of steroidal compound is 5α-cholestane-3, 6-dione (LIII). This compound was prepared, characterized and its detail study of X-ray crystallography was done. The results are : molecular formula, $C_{27}H_{44}O_2$; molecular weight, 400; crystal morphology; colourless plate; crystal dimensions (mm); 0.20x0.50x0.30, crystal system; monoclinic, lattice parameters; a = 8.216 (3), b = 7.616 (2), c = 19.706 (3) A°, β=92.86 (2), volumes; 1231.6 (5) $A°^3$, space group; $P2_1$, (≠4), Z value; 2 (two molecules per unit cell), radiation ; Cu Kα = 1.5417 A°, temperature; 23° C, structure solution; direct method.

In the structure of 5α-cholestane-3, 6-dione (LIII) (Fig. 9a, b) C3 and C6 are Sp^2 – hybridized carbon atoms. Due to this the respective portions (C2, C3, C4 and C5, C6 C7) acquiring planority cause definite conformational deformity in both rings A and B, their conformation became pseudo chair form.

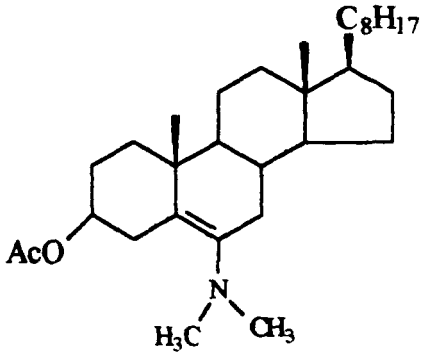
CHAPTER-FIVE

Neurotoxicological Effects of Steroidal Compounds on Lipid Metabolism in Different Regions of Rat Brain :

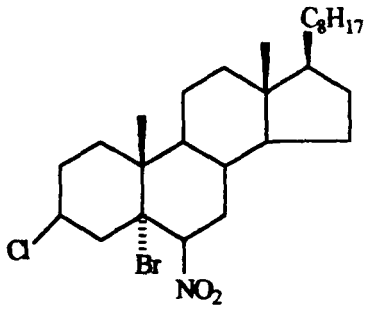
Steroidal compounds 3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane (LIV), 3β-acetoxy-6-dimethylamino cholest-5-ene (LV), 3β-chloro-5-bromo-6β-nitro-5α-cholestane (LVI) and 6β-aminopyrimidino cholest-4-en-3-one (LVII) were designated as A, B, C and D respectively. The present study was under taken to evaluate the neurotoxic effects of these steroidal compounds on lipid metabolism.



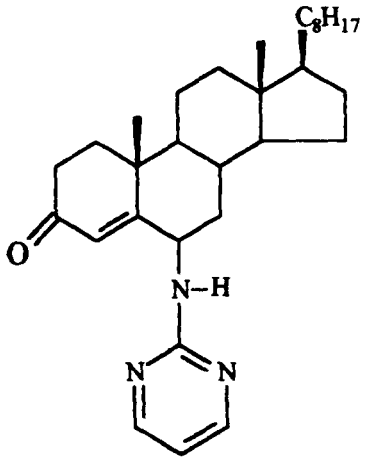
3β-Acetoxy-5-bromo-6β-hydroxy-5α-cholestane (LIV) (A)



3β-Acetoxy-6-dimethylamino cholest-5-ene (LV) (B)



3β-Chloro-5-bromo-6β-nitro-5α-cholestane (LVI) (C)

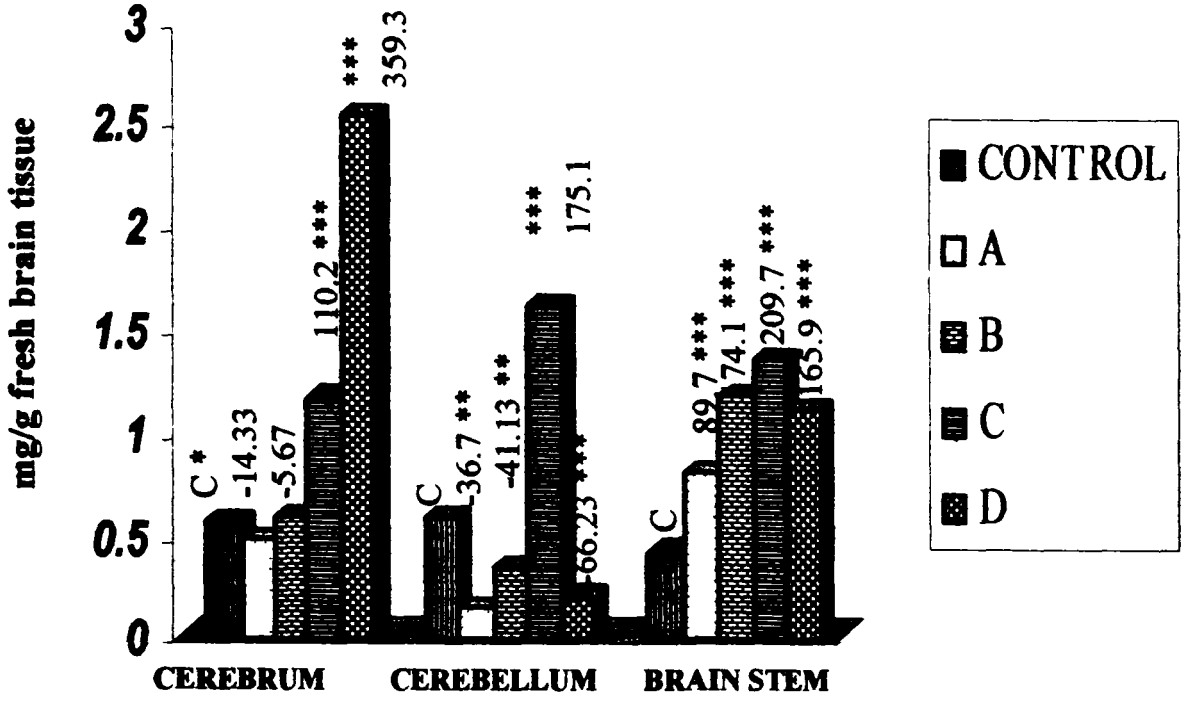


6β-Aminopyrimidino-cholest-4 en-3-one (LVII) (D)

This study shows the effects of four steroidal derivatives (3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane(LIV) (A); 3β-acetoxy-6-dimethylamino-cholest-5-ene (LV) (B); 3β-chloro-5-bromo-6β-nitro-5α-cholestane (LVI) (C); 6β-aminopyrimidino-cholest-4-en-3-one (LVII) (D) on the total lipids concentration in rats brain. The 3β-chloro-5-bromo (LVI) (C) produces significant increase of 110.2% in the cerebrum and 175.1% in the cerebellum, while most significant increase of 209.7% in the total lipid contents is observed in brain stem.

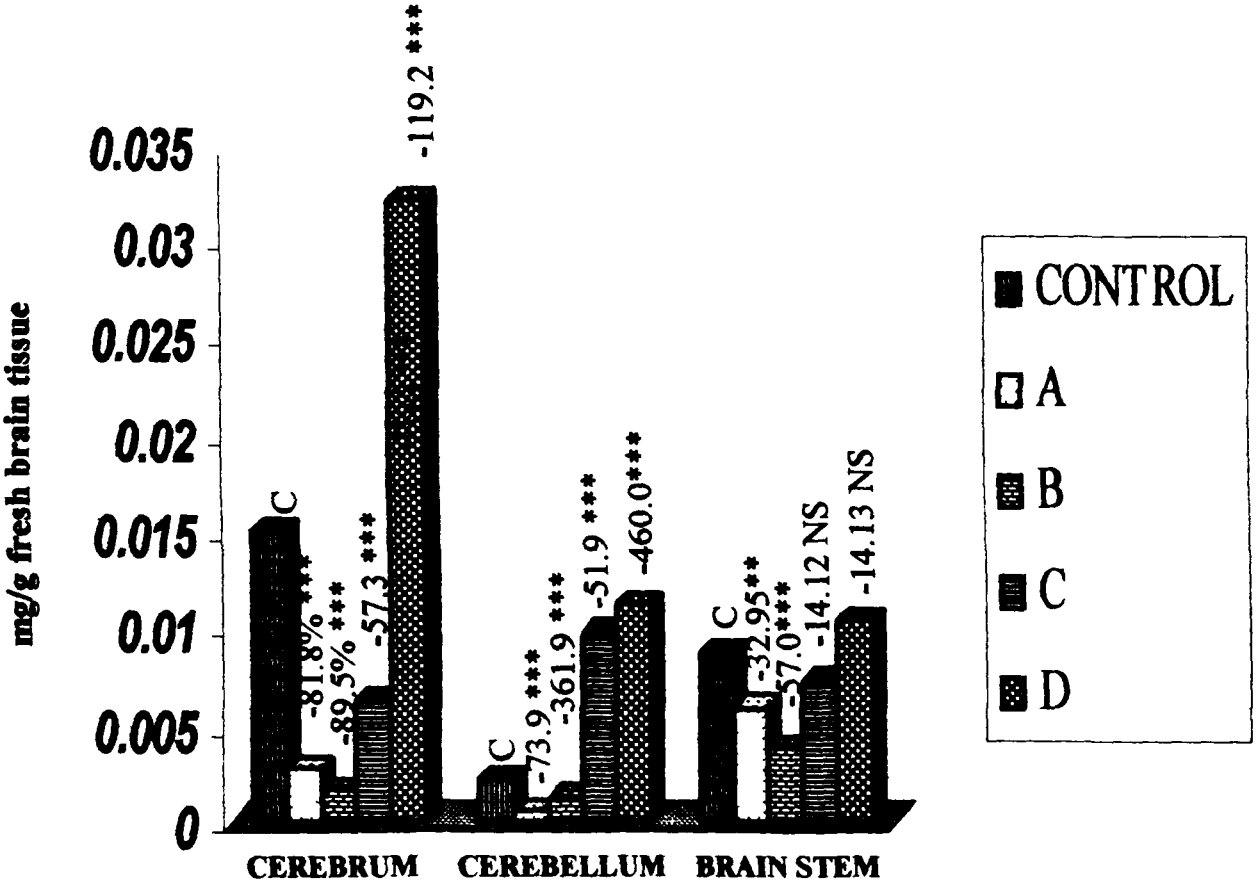
The 6β-Aminopyrimidinocholest-4-en-3-one (LVII) (D) produces significant increase 165.9% and most significant increase of 359.3% in the concentration of total lipids in brain stem and cerebrum respectively while significant decrease of occurs in brain stem –66.23%.

The 3β-acetoxy-5-bromo-6β-hydroxy-5α (LIV) (A) produced significant decrease of -36.7% and significant increase 89.7% in the concentration of total lipids in cerebellum and brain stem and it shows insignificant decrease of –14.33% in cerebrum respectively. The compound (LV) (B) shows significant increase 174.1% and significant decrease of –41.13% in the concentration of total lipids in brain stem and cerebrum respectively while insignificant decrease of –5.67% occur in brain stem.



(Fig. 10)
Histogram of Total Lipids

In the content of cholesterol it shows that 6β-aminopyrimidino (LVII) (D) produces most significant increase 460.0% in cerebellum, while significant decrease is observed –119.2% of cerebrum and 14.13% in brain stem. 3β-Chloro-5-bromo-6β (LVI) (C) produces significant decrease – 361.9% in the concentration of cholesterol in cerebellum and –57.3% in cerebrum, while it produces an insignificant deprivation in brain stem - 14.12%. 3β-Acetoxy-5-bromo (LV) (B) produces significant decrease - 89.7% in cerebrum, -57.8% in brain stem and –51.9% in the concentration of cholesterol in cerebellum. 3β-Acetoxy-5-bromo-6β-hydroxy (LIV) (A) produces significant decrease in the cholesterol level in cerebrum -81.8% in cerebellum -73.9% and in brain stem -32.9%.

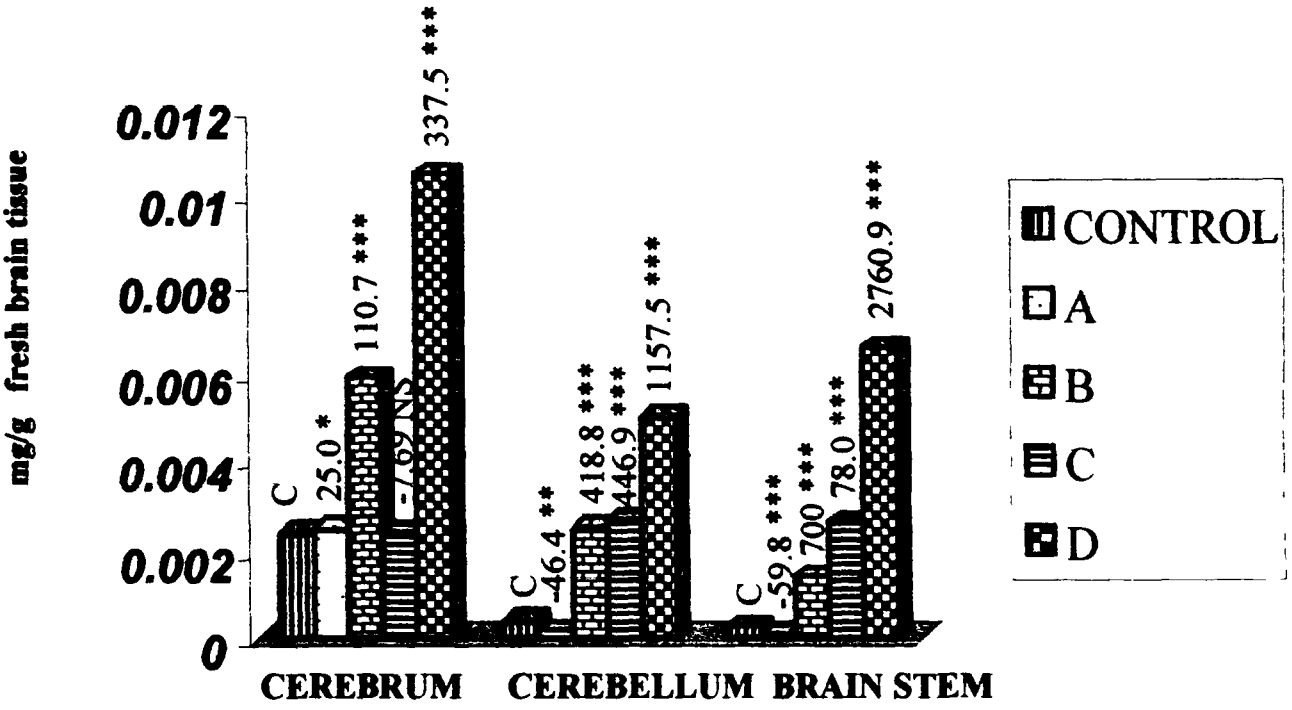


(Fig. 11)

Histogram of Cholesterol

The steroidal derivative 6β-aminopyrimidino (LVII) (D) produces most significant increase in the level of gangliosides in brain stem (2760.9%) and cerebellum (1157.5%) and of cerebrum (337.5%). 3β-chloro-5-bromo (LVI) (C) produces most significant increase in level of gangliosides in brain stem (780.0%), cerebellum (446.9%) and insignificant decrease in cerebrun (-7.69%) is observed. 3β-acetoxy-6-dimethylamino (LV) (B) produces most significant increase in brain stem (700.0%), cerebellum (418.8%) and in cerebrum (110.7%). 3β-acetoxy-5-bromo-6β (LIV) (A) produces significant

decrease in level of gangliosides in brain stem (-59.8%) and in cerebellum (-46.5%). While significant increase is observed (25.0%) in cerebrum.



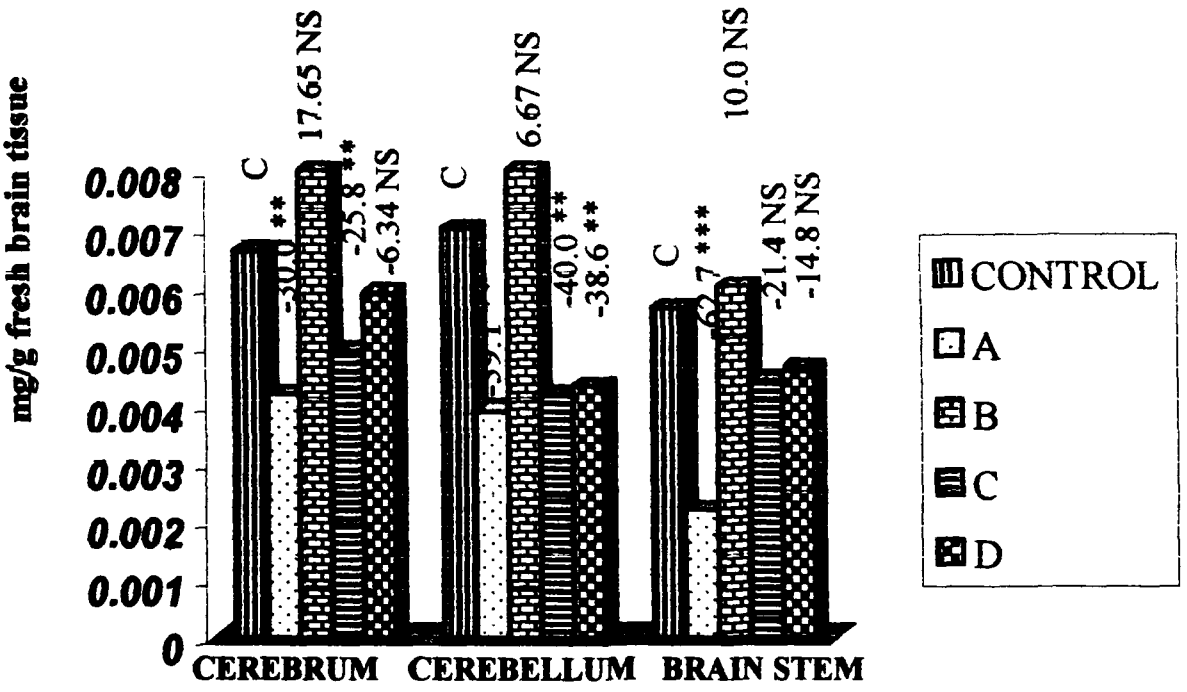
(Fig. 12)

Histogram of gangliosides

The data obtained for the rate of lipid Peroxidation following the administration of four steroidal derivatives. 3β-Acetoxy-5-bromo-6β-hydroxy (LIV) (A) produces significant decrease of 62.7% in brain stem, -39.1% in cerebellum and of cerebrum (-30.0%) is observed.

3β-acetoxy-6-dimethylamino (LV) (B) produces insignificant increase of 17.65% in cerebrum, 10.0% in brain stem and 6.67% is reported in cerebellum of rats brain. 6β-Aminopyrimidino (LVII) (D) produces significant

decrease of –38.6% in cerebellum while insignificant depletion of 14.8% and 6.34% is found in brain stem and cerebrum respectively. 3β-Chloro-5-bromo (LVI) (C) produces significant decrease of 40.0% in cerebellum and of 25.8% in cerebrum. While insignificant decrease of 21.4% is reported in brain stem of rats brain in the study.



(Fig. 13)

Histogram of lipid peroxidation

These steroidal compounds were prepared according to literature procedure characterized by spectral and chemical evidences and comparison with authentic sample in known cases.

SYNTHESIS, CHEMICAL, BIOCHEMICAL, X-RAY AND OTHER SPECTRAL STUDIES OF MODIFIED STEROIDS

THESIS SUBMITTED FOR THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

Mrs. SHEEBA SHAFI

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2000

Dr. M. Mushfiq
Professor of Chemistry

Tel. : 0571-400515

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH

Date 7.7.2000

This is to certify that the work embodied in thesis entitled ***"Synthesis, Chemical, Biochemical, X-Ray and other Spectral Studies of Modified Steroids"*** is the original work done by ***Mrs. Sheeba Shafi*** under my supervision. The thesis is suitable for submission for the award of the degree of Doctor of Philosophy in Chemistry.

(Dr. M. Mushfiq)

Res. B-2, M.I.G. Flats, Diggi Road, Aligarh-202001 (U. P.), INDIA Tel. 0571-401726

Dedicated

To

Jb. Ibrar Hussain
Khan 'Alvi'

My enlightened Father - in - Law.

Mrs. Sheeba Shafi
Steroid Research Laboratory
Department of Chemistry
A.M.U., Aligarh – 202002
(U.P.) - INDIA

Tel : 400515 (Deptt.)
502247 (Res.)
E-Mail: chr008@amu.up.nic.in

Acknowledgement

With utmost pleasure and privilege I am endeavoring to express my sincere obligations to my supervisor ***Prof. M. Mushfiq****, Deptt. Of Chemistry AMU Aligarh. I am fortunate to have his connoisseur guidance, his cooperation, his helpful discussions and his modern sense of scientific approach and constant encouragement which enabled me to successfully complete this work. He has always been a well wisher of me, guided every step of my carrier since school days, treated me as a child and taught me abc of chemistry. I will remain obliged life long and highly indebted for his personal attention and academic competence, which will always be a source of inspiration to me.*

I am highly grateful to my husband ***Dr. Ehtsham Hussain Khan*** *for his ever extending help, unending support and his caring nature and loving approach toward me proved a boon which provided intensive strength and boosted my moral at every step of this study. It is he, who always dragged me to work more and more and always encouraged me*

to become a productive researcher. I am thankful to Allah to have such kind and affectionate husband who always stand by me to improve my personality.

I will be failing in my duty if I will not acknowledge the invaluable kind and generous support of ***Late Prof. M. S. Ahmed*** *– the treasure of chemistry for his unstined generous guidance and advices throughout my academic carrier from my childhood. He was not only a highly modest and erudite person but pride of India in the field of chemistry. His life is source of inspiration to me which teaches me to work hard, gain more and more knowledge and lead a disciplined life.*

It is my privilege to thank ***Prof. M. Ilyas*** *Department of Chemistry A.M.U., Aligarh, who has always given fatherly treatment throughout my carrier since my childhood and always provided his highest expertise and efficient guidance during completion of my work.*

I sincerely thank ***Professor Saeuduzzafer Quershi****, Chairman Department of Chemistry, A.M.U., Aligarh, for providing necessary research facilities. I am highly thankful to present director of IBRC, J. N. Medical College,* ***Prof. Shamin Jahan Rizvi*** *for the generous support during the Biochemical portion of my work I am equally thankful to my*

hospital collegues ***Dr. Fakrul Islam, Dr. Kr. Asif, Mr. Farroque, Miss Uzma, Mr. Umar*** *and* ***Mr. Istiaque*** *for their help during biochemical part of my thesis.*

I am sincerely acknowledging it with a lot of thanks that the timely help encouragement and invaluable suggestions from ***Dr. Hasrat Ali****, Research Professor Sheerbrooke University, Canada which help me to complete this research work. My deepest thanks and appreciation to* ***Dr. Hasrat Ali*** *and* ***Takayangi****, Canda and Japan respectively for his outstanding contribution for spectral recording.*

I am proud of mentioning the name of my well ***wisher friend Mrs. Zoya Samar*** *she is just like a sister to me and always encouraged me at every step of life.*

I can never forget mentioning the names of my lab colleagues, ***Dr. Mehtab Parveen, Mrs. Afreen Shamim, Miss. Rakshanda Rahman, Miss Nazish Siddiqui, Dr. Jamal Firoz, Mr. Salman Hashmi, Mr. Jamal Ahmad, Mr. Mehboob*** *and* ***Mr. Aftab*** *for their constant encouragement and valuable cooperation.*

Above all, my sincere thanks to my loving family members, my mummy, ***Mrs. Mushtar Jahan****, she is a wonderful mother and nearest*

friend of mine, her love, care and affection has build permanent place for her in the deepest core of my heart, her support and encouragement is really priceless and beyond the expectation and a debt upon me for whole life. She is indeed the person nearest to my heart and soul I am equally grateful to my mother in law ***Mrs. Noor Jahan****, who consider me as her daughter and always take care of me as a mother she is one of the most lovable person I have ever met in my life.*

I am proud of mentioning the name of my father–in–law ***Jb. Ibrar Hussain Khan Alvi*** *it is his support and encouragement which boosted my moral and helped me to stride high, increased my will power and always stood by me throughout this tedious task. It is he without whose cooperation this study was impossible for me. He is just like a father to me and has always treated me as a daughter. He is highly dynamic, innovative, modest person and deals both family and society very efficiently with ever smiling face.*

It will be unjust onpart of me if I will not mention special acknowledgement and gratitudes to my sister–in–law ***Roshan*** *and brother–in–law* ***Intakhab Bhai, Tanveer, Taqueer Alam Khan****.*

In last but not the least my special thanks to my sisters ***Kahkshan, Uzma, Anjum, Saba, Mrs. Shadma, Shabnam, Azra Khan*** *and* ***GhazalaKhan*** *and to my brothers* ***Fizauddin Khan, Sharfuddin Khan, Arshuddin Khan, Saluddin Khan, Shoib, Saleem*** *and* ***Mohiuddin.***

My thanks to ***Mr. Tariq*** *and* ***Mr. Ayub Khan*** *for typing the manuscript with patience.*

Sheeba Shafi

Sheeba Shafi

CONTENTS

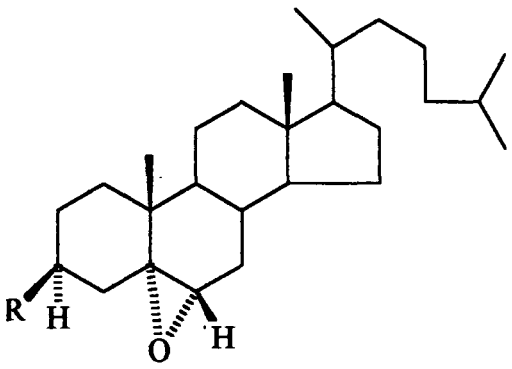
SUMMARY

The chemistry of steroids is a matter of great interest because of their immense use in research and industry owing to their broad spectrum of biological properties. In the thesis, the synthesis of some important hetero steroids are described. The compounds synthesized were characterized on the basis of chemical, analytical and spectral evidences. Biochemical studies of some of them has been done. The results were summarized chapterwise as below.

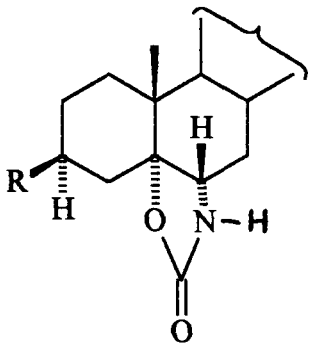
CHAPTER-ONE

Synthesis of Steroidal Oxazolines and Aziridines (Reaction of Steroidal Epoxides) :

Reaction of steroidal epoxides with appropriate reagents leading to the synthesis, of steroidal oxazolidines, oxazoles, oxazolines, oxathiolanethiones and aziridine have been reported from our laboratories and from other research centres because of their pharmaceutical importance which include inflammatory, hypertensive, tranquilizing and carcinostatic activities. Recent publication from our laboratories deals with the synthesis of steroidal oxazolidinones (IV-VI) which involved reactions of steroidal epoxides (I-III) with glycine.

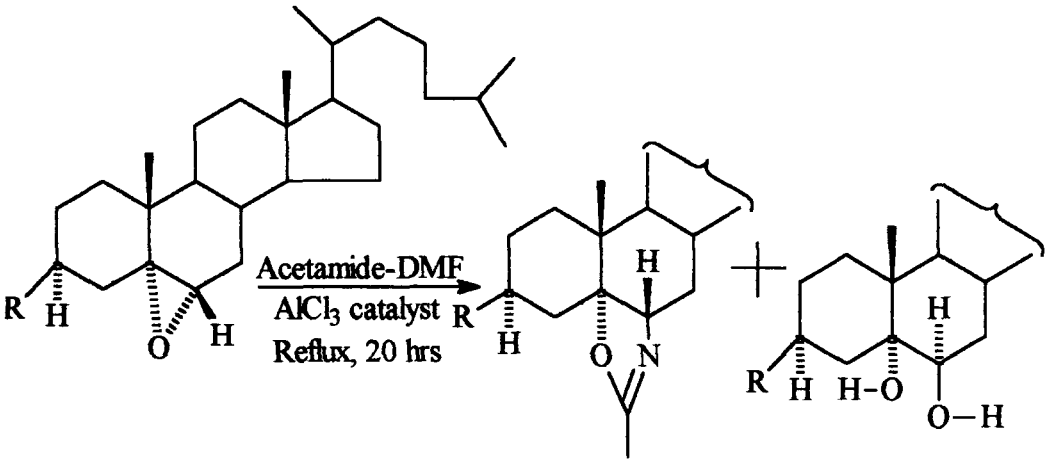


	R
(I)	H
(II)	Cl
(III)	OAc



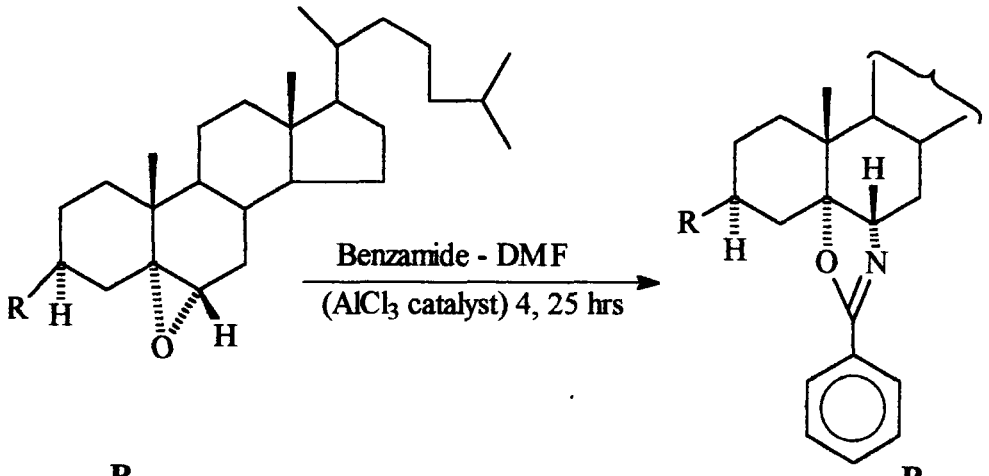
	R
(IV)	H
(V)	Cl
(VI)	OAc

In continuation to our study related to the synthesis of steroidal compounds containing heterocyclic ring, new steroidal oxazolines and aziridines in cholestane series were reported in this chapter, when 5,6α-epoxy-5α-cholestane (I), its 3β-chloro (II) and 3β-acetoxy (III) analogues were treated with actetamide in DMF ($AlCl_3$ as catalyst) at reflux condition for 20-25 hrs. afforded 5α-cholestano[5,6α-d]-2′-methyl-2-oxazoline (VII), its 3β-chloro (VIII) and 3β-acetoxy (IX) analogues along with 5,6β-dihydroxy-5α-cholestane (X), its 3β-chloro (XI) and 3β-acetoxy (XII) analogues.



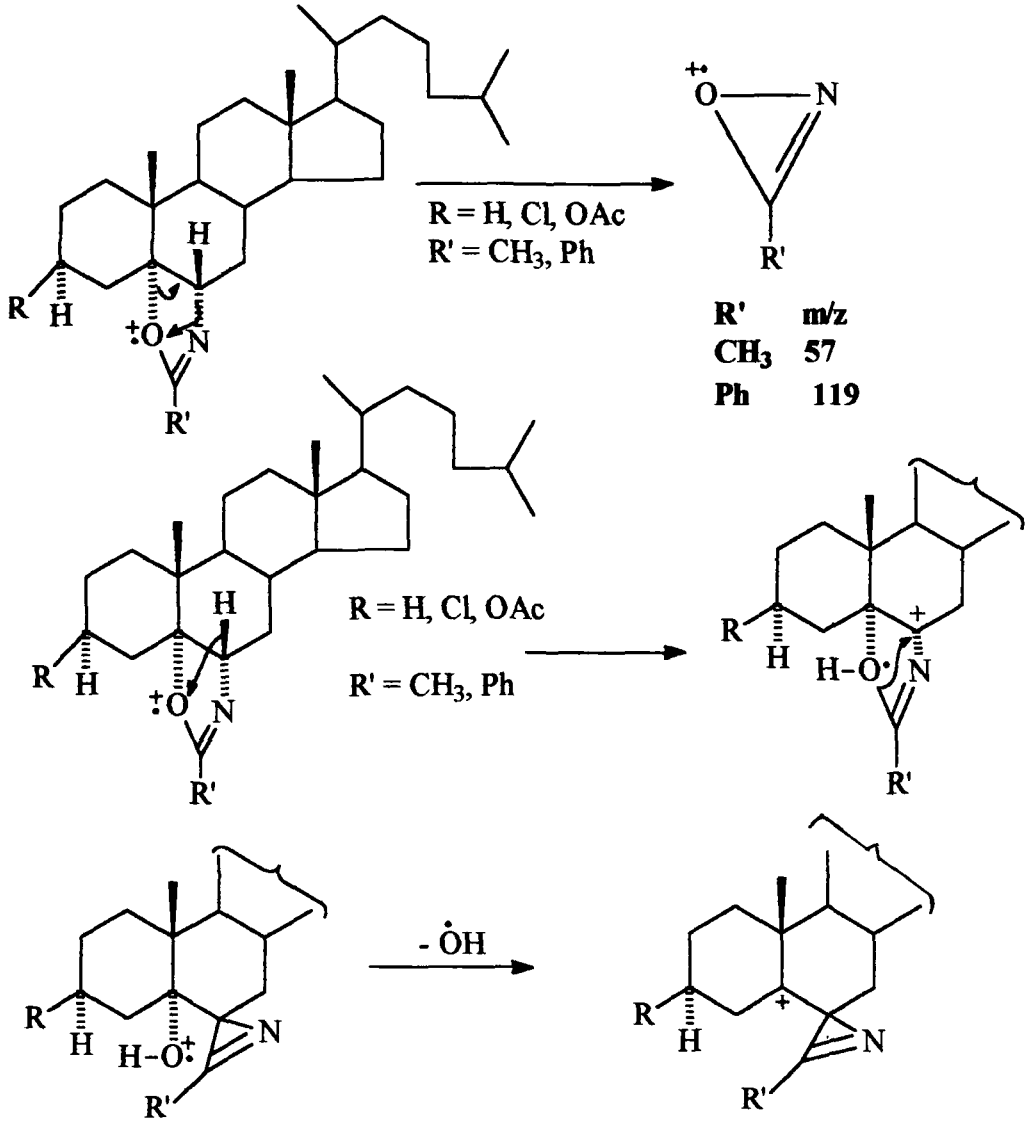
R	R	R
(I) H	(VII) H	(X) H
(II) Cl	(VIII) Cl	(XI) Cl
(III) OAc	(IX) OAc	(XII) OAc

When the same reaction was repeated with benzamide – DMF ($AlCl_3$ a catalyst, reflux for 25 hrs.) with steroidal epoxides (I – III), 5α-cholestano [5, 6α-d]-2′-phenyl-2-oxazoline (XIII) its 3β-chloro (XIV) and acetoxy (XV) analogues were obtained along with the steroidal diols (X – XII).



R	R
(I) H	(XIII) H
(II) Cl	(XIV) Cl
(III) OAc	(XV) OAc

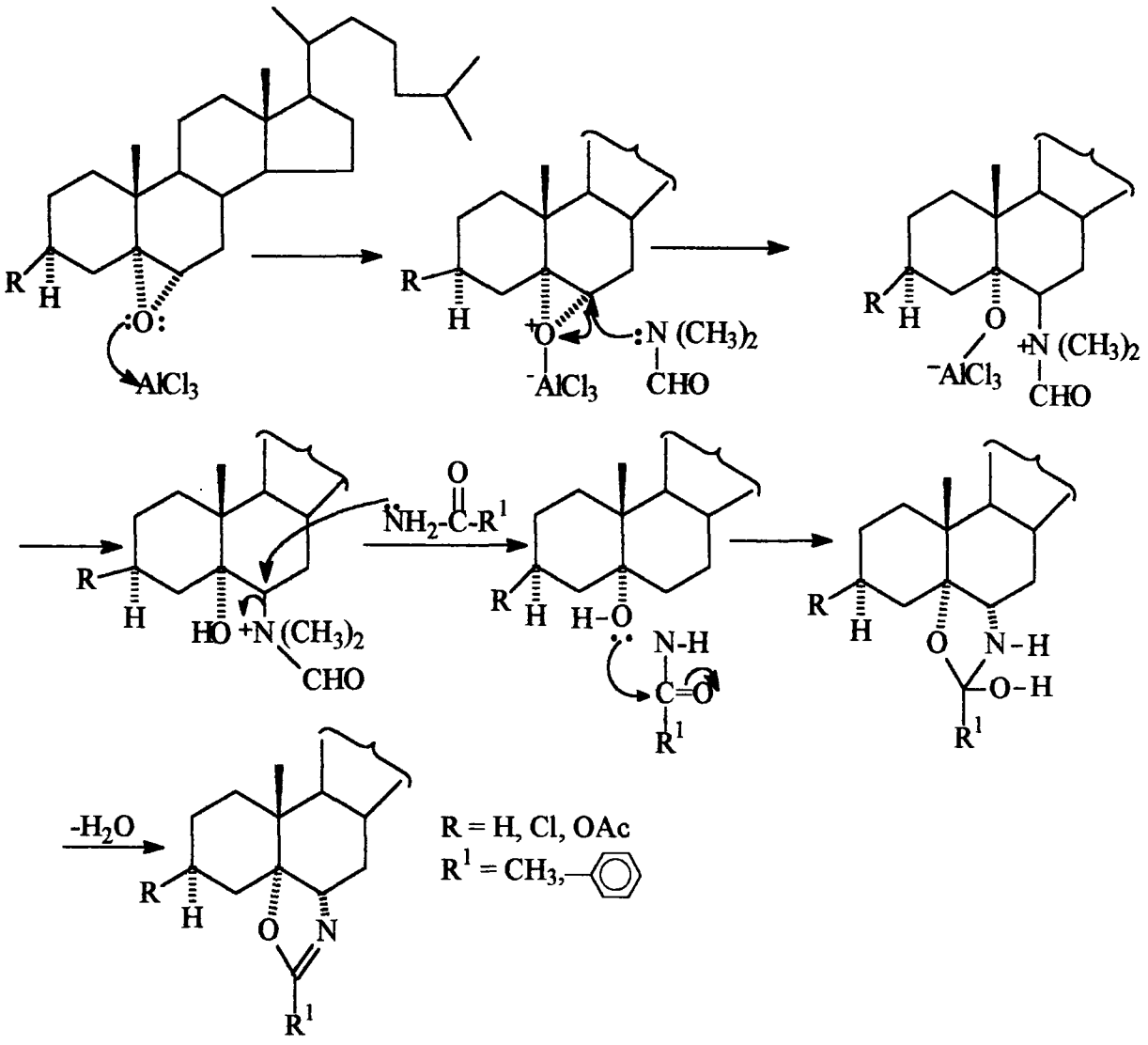
The structure of steroidal oxazolines (VII – IX) and (XIII – XV) was established on the basis of analytical and spectral evidences. Mass spectral studies has given strong support for the assigned structure. Two diagnostic fragment ions obtained were given in scheme – 1.



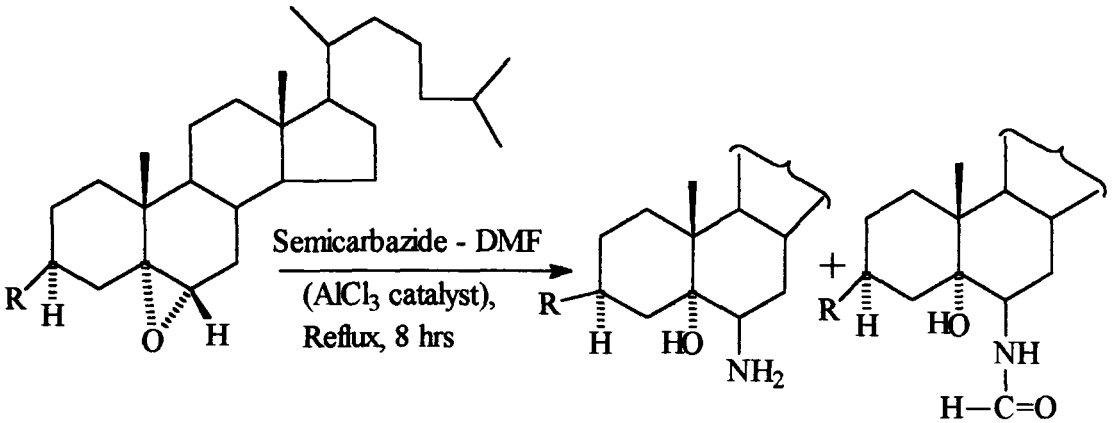
R	R'	M/z	R	R'	m/z
H	CH_3	410	H	Ph	460
Cl	CH_3	444 / 446	Cl	Ph	506 / 508
OAc	CH_3	457	OAc	Ph	530

Scheme - 1

A mechanism has been written to explain the formation of steroidal oxazolines (VII – IX) and (XIII – XV) in which double SN^2 – inversion on epoxide ring carbon (C6) has occurred. Formation of diols (X – XII) was because of the trans diaxial ring opening of epoxides (I – III).



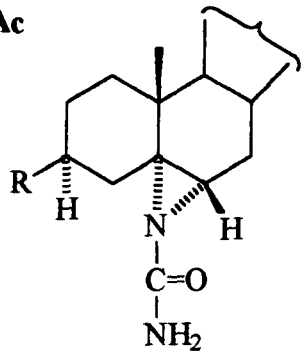
When 5, 6α-epoxy-5α-cholestane (I), its 3β-chloro (II) and 3β-acetoxy (III) were treated with semicarbazide in DMF ($AlCl_3$ as catalyst) afforded 5-hydroxy-6β-amino-5α-cholestane (XVI), 5-hydroxy-6β-amino-N-formyl-5α-cholestane (XIX) and N-amido-5α-cholestano [5, 6-b]-aziridine (XXII) and their corresponding 3β-chloro (XVII, XX, XXIII) and acetoxy (XVIII, XXI, XXIV) analogues.



	R
(I)	H
(II)	Cl
(III)	OAc

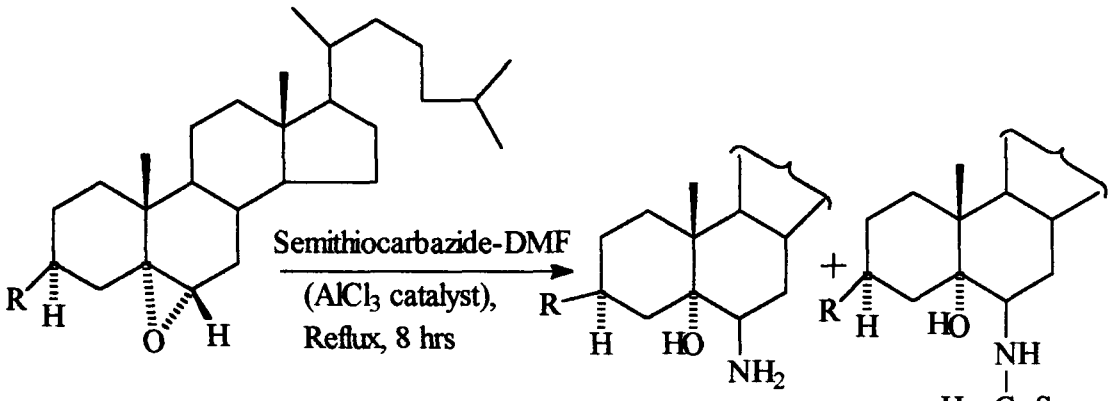
	R
(XVI)	H
(XVII)	Cl
(XVIII)	OAc

	R
(XIX)	H
(XX)	Cl
(XXI)	OAc



	R
(XXII)	H
(XXIII)	Cl
(XXIV)	OAc

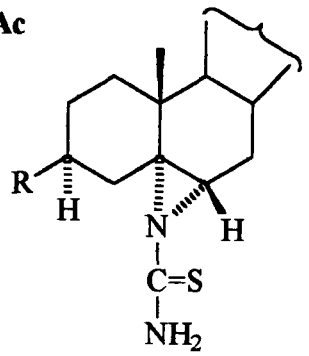
When the same epoxides (I–III) were treated with phenyl semicarbazide under identical reaction conditions same products (XVI – XVIII). (XIX – XXI) and (XXII – XXIV) were obtained. The steroidal epoxides (I – III) when treated with semithiocarbazide under same reaction conditions afforded hydroxyamino compounds (XVI – XVIII), aminothioformyl compounds (XXV- XXVII) and thioamido aziridines (XXVIII – XXX).



R	
(I)	H
(II)	Cl
(III)	OAc

R	
(XVI)	H
(XVII)	Cl
(XVIII)	OAc

R	
(XXV)	H
(XXVI)	Cl
(XXVII)	OAc

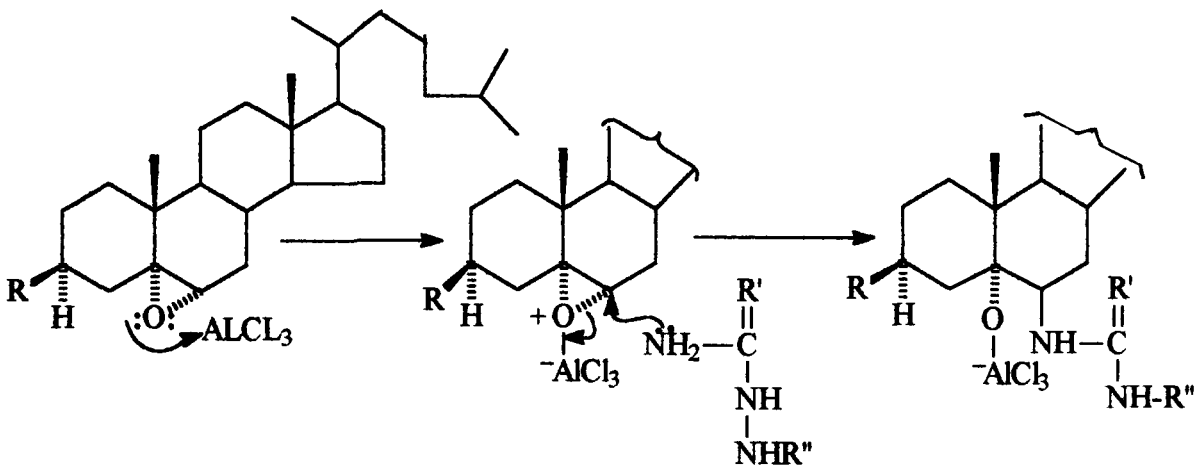


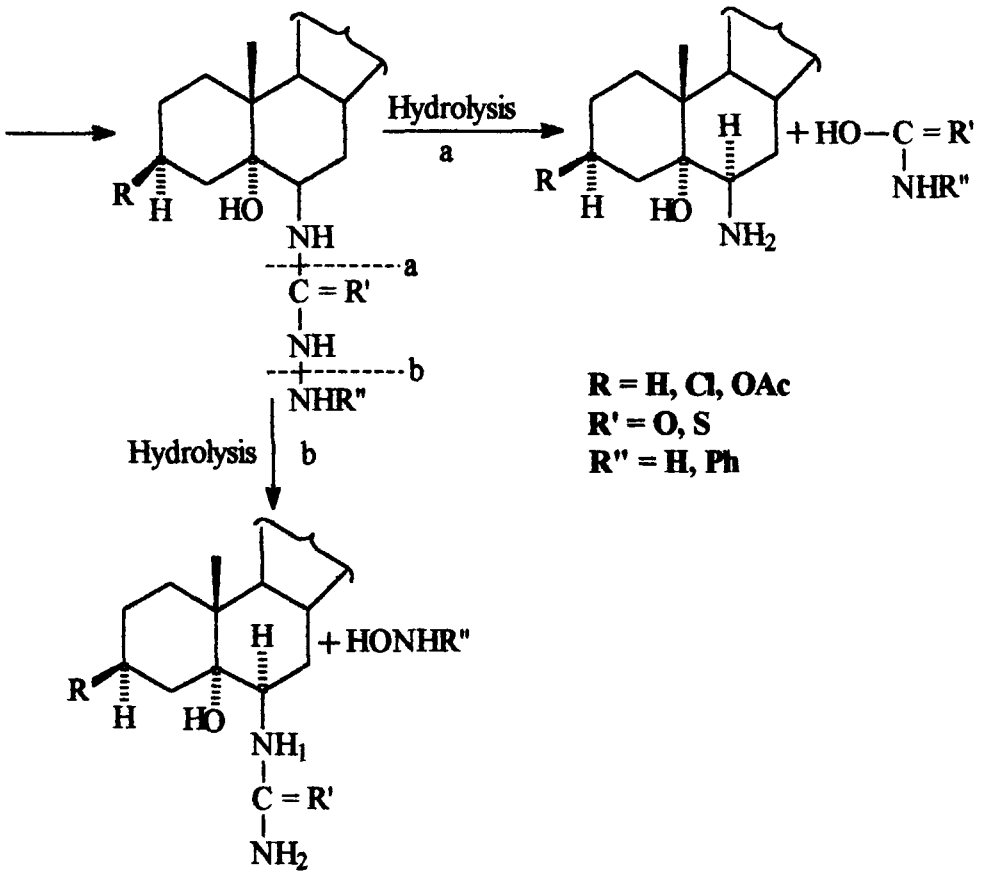
R	
(XXVIII)	H
(XXIX)	Cl
(XXX)	OAc

The structure of these amino steroids was confirmed on the basis of analytical and spectral (IR, ^{1}H-NMR and Mass) evidences. Formation of these compounds was explained on the basis of mechanism (1 and 2).

Mechanism – 1 :

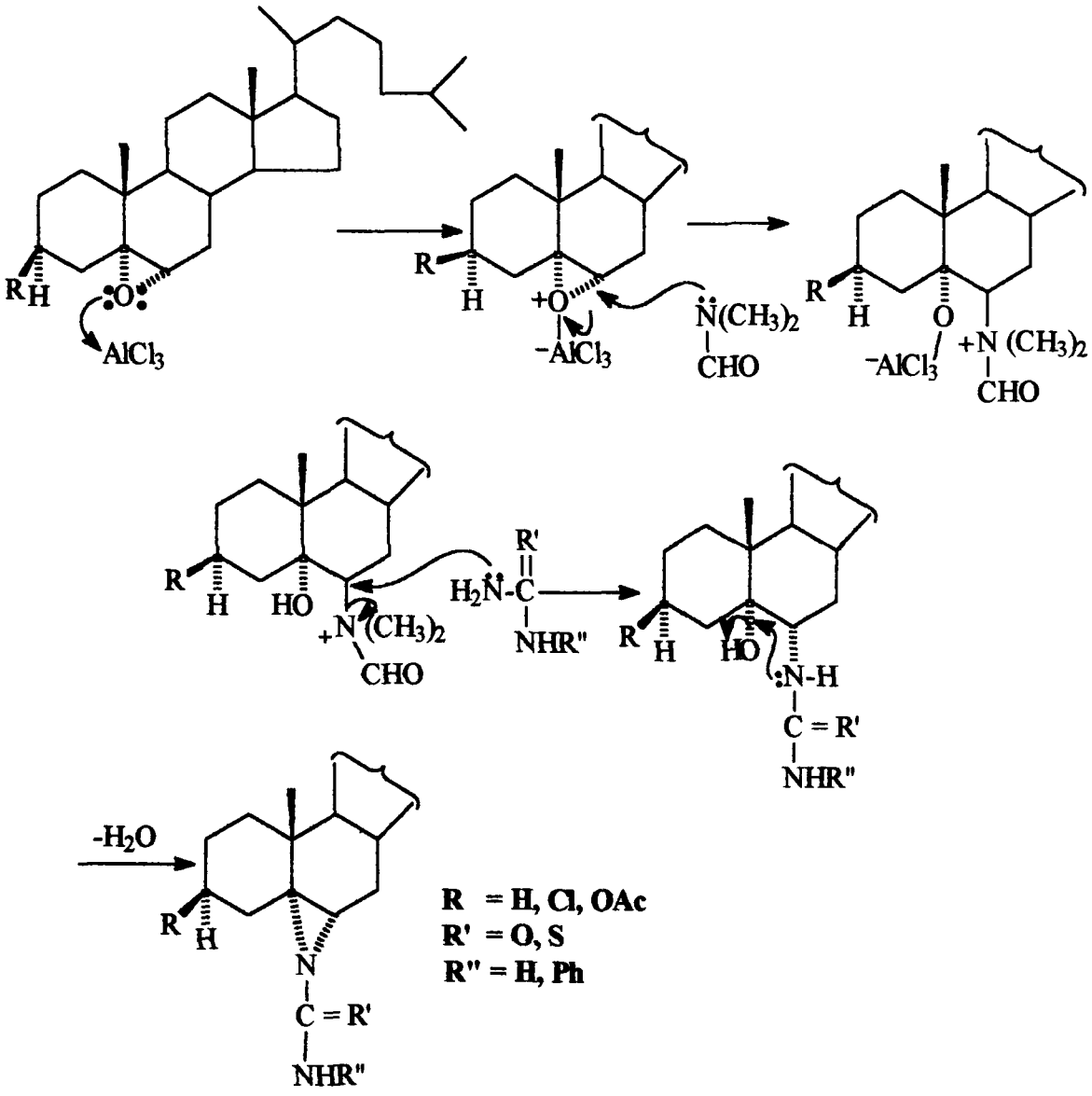
Formation of hydroxy amino compounds and amino formyl and thio formyl compounds were explained as follows where during hydrolysis via path a provided hydroxyamino compounds where as path b afforded amino formyl and thio formyl compounds.





Formation of amidoaziridines and thioamidoaziridines can be explained on the basis of mechanism – 2. In which double SN^2 – inversion on epoxide ring carbon (C6) has occurred.

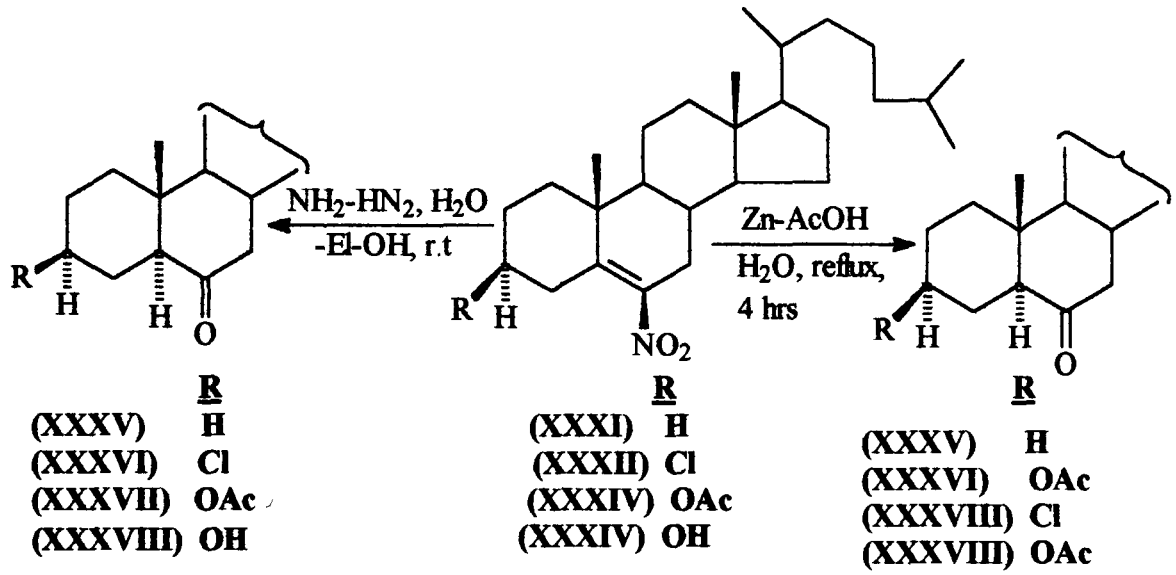
Mechanism – 2 :


R
H
:O:
AlCl3
+O
-AlCl3
N(CH3)2
CHO
O
+N (CH3)2
HO
+N (CH3)2
H2N-C
R'
NHR"
:N-H
C = R'
-H2O
N
R = H, Cl, OAc
R' = O, S
R" = H, Ph

CHAPTER-TWO

Reduction of Vinyl Nitrosteroids :

In the recent past synthesis of steroidal compounds have gained importance because of biological activities associated with them. Reduction is one among the various reactions used in the synthetic pathway leading to important steroidal compounds. Many types of reagents which have been successfully employed for reduction are hydrogen with metal, lithium alumininum hydride, zinc acetic acid, sodium borohydride and other metallic hydrides. Photochemical and electrochemical methods were also employed for reduction. Raney Nickel catalysed hydrazine - hydrate reduction has not been studies thoroughly. In this chapter a comparative study has been made between zinc–acetic acid and hydrazinc-hydrate (Raney Nickel catalysed) reduction of vinyl nitrosteroids. 6-nitrocholest-5-ene (XXXI), its 3β-acetoxy (XXXII), 3β-chloro (XXXIII) and 3β-hydroxy (XXXIV) analogues were subjected to both zinc-acetic acid and hydrazine-hydrate (Raney Nickel) catalysed reduction.

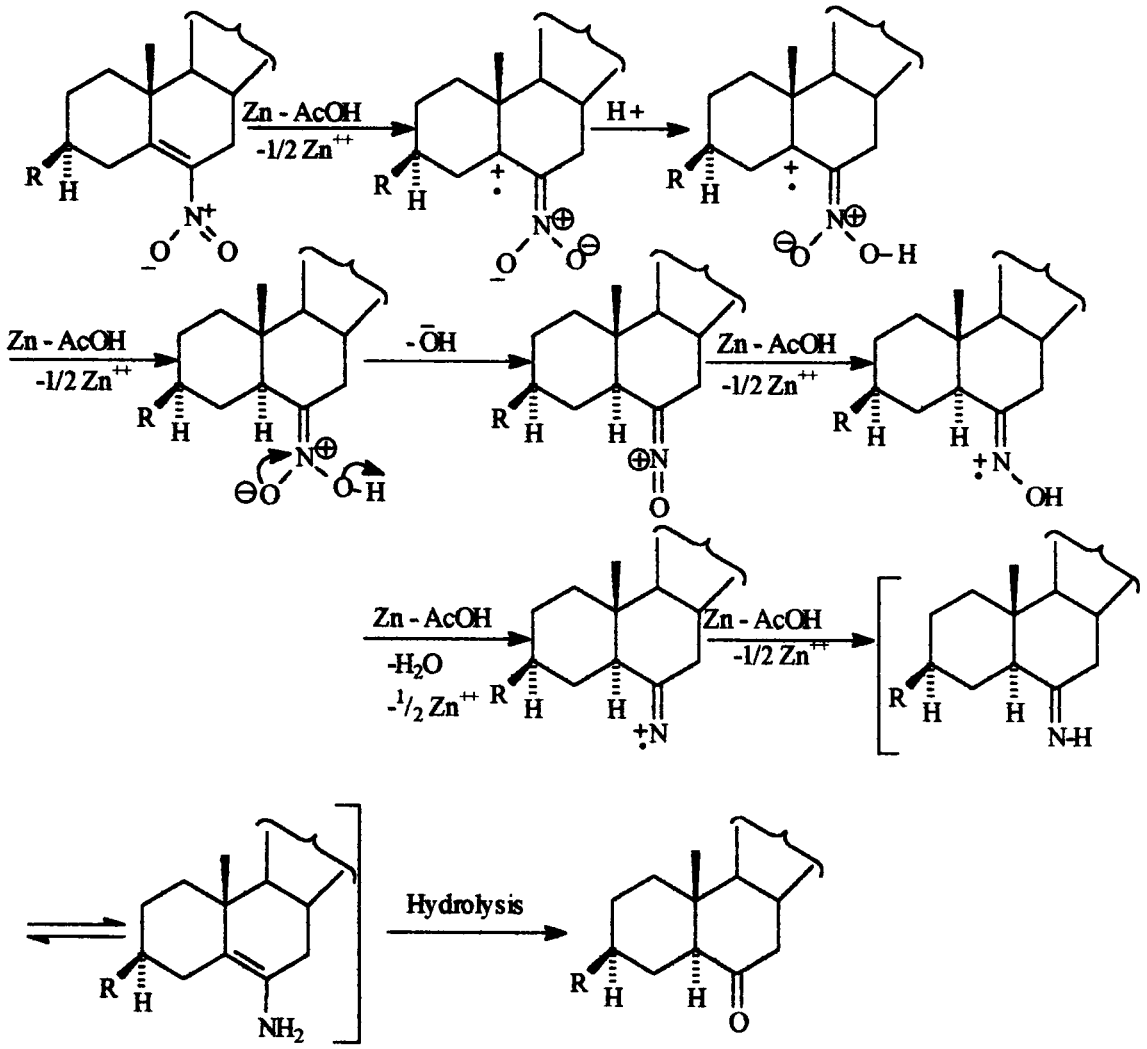


R	R	R
(XXXV) H	(XXXI) H	(XXXV) H
(XXXVI) Cl	(XXXII) Cl	(XXXVI) OAc
(XXXVII) OAc	(XXXIV) OAc	(XXXVIII) Cl
(XXXVIII) OH	(XXXIV) OH	(XXXVIII) OAc

Both the reduction methods gave the same ketones with respect to the vinyl nitrosteroids but the reaction conditions are mild and yield is better in hydrazinc-hydrate (Raney Nickel) catalysed reduction. The mechanism of reduction is proposed in both the cases.

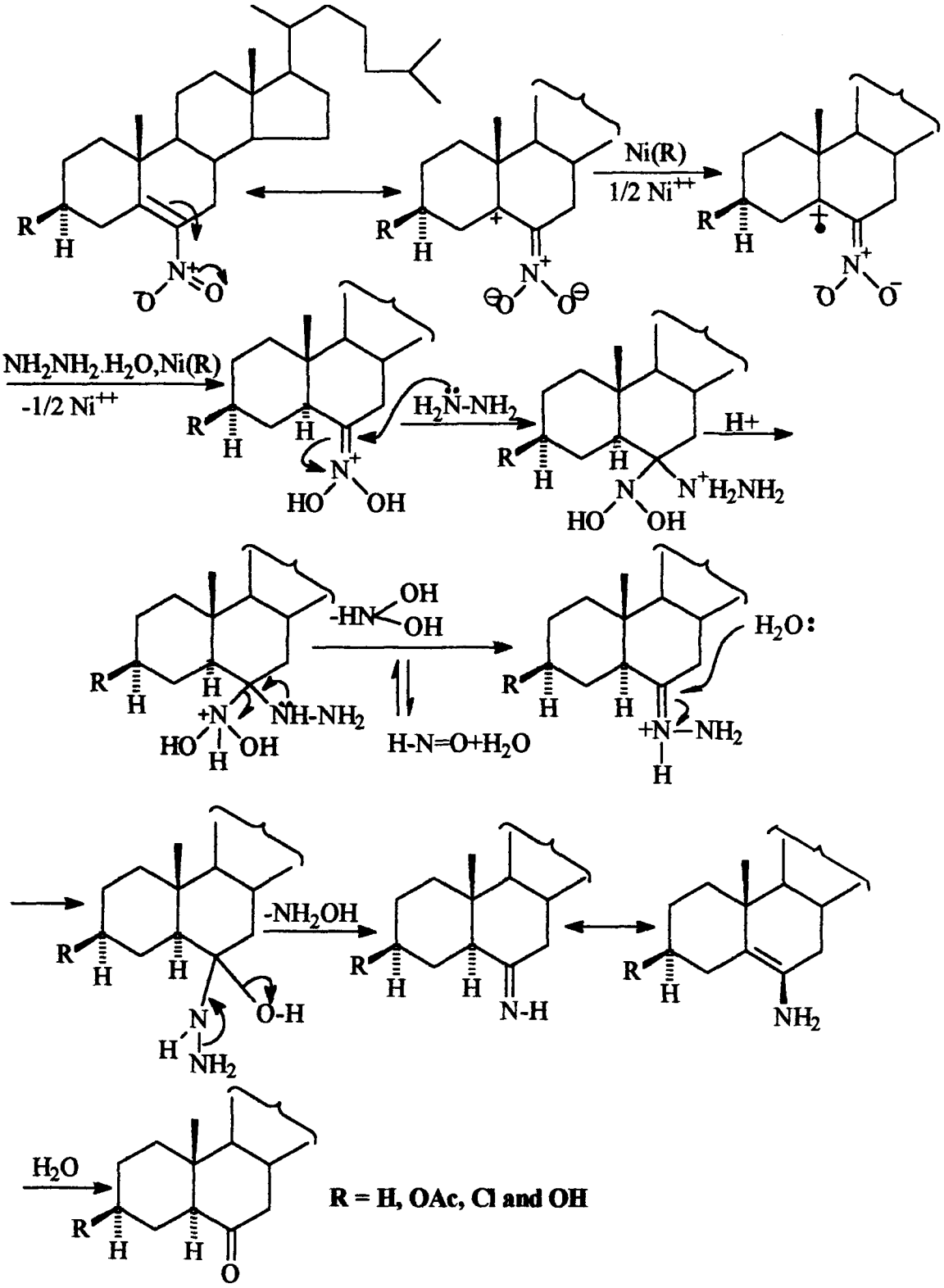
Zn-AcOH Reduction of Vinylnitro Steroids :

Mechanism :



Hydrazine – Hydrate (Raney Nickel Catalysed) Reduction :

Mechanism


Ni(R)
1/2 Ni++
NH2NH2.H2O,Ni(R)
-1/2 Ni++
H2N-NH2
H+
-HN(OH)OH
H2O:
H-N=O+H2O
-NH2OH
H2O
R = H, OAc, Cl and OH

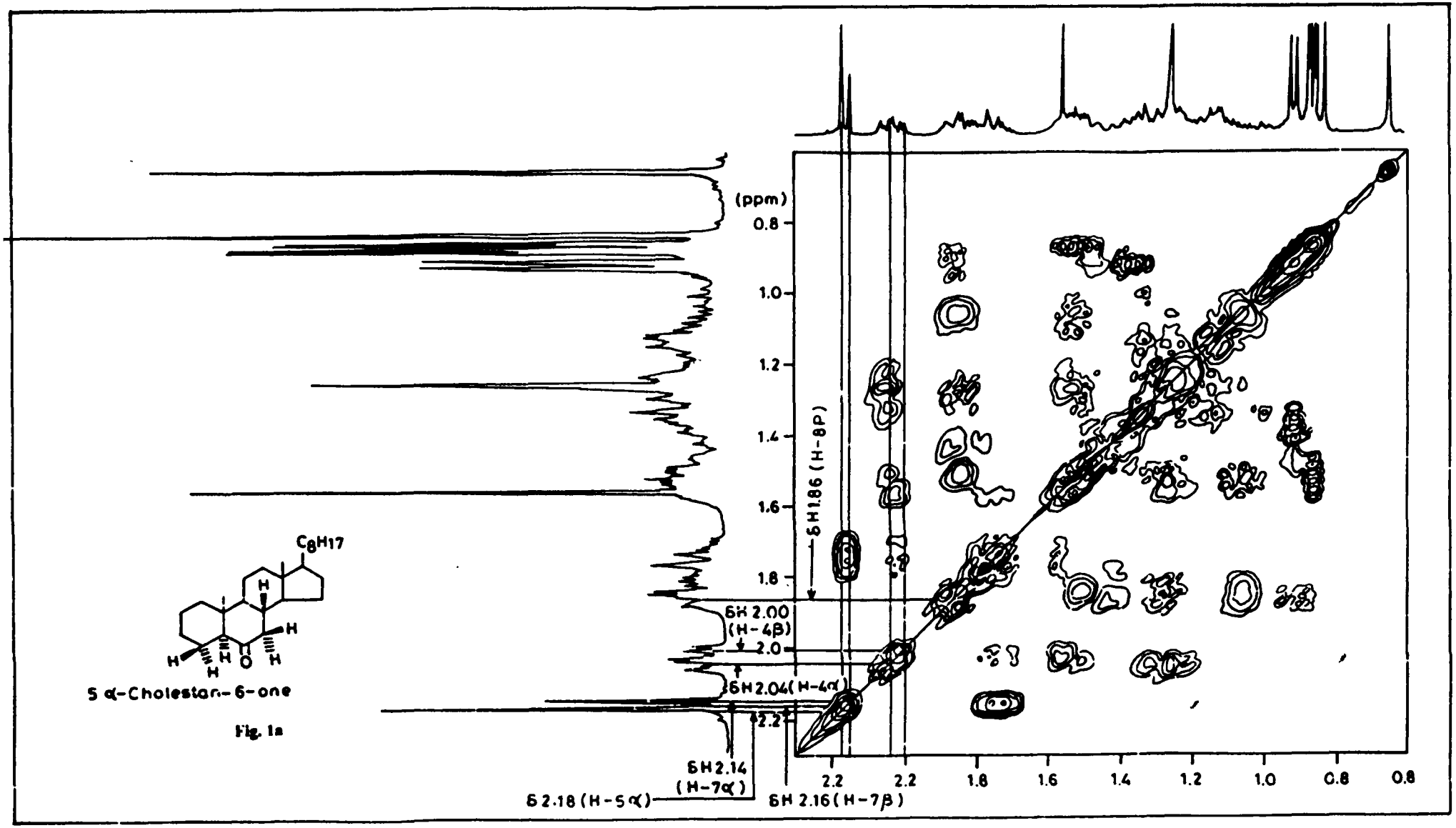

(ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
δH 1.86 (H-8β)
δH 2.00 (H-4β)
δH 2.04 (H-4α)
δH 2.14 (H-7α)
δ 2.18 (H-5α)
δH 2.16 (H-7β)
2.2
2.2
1.8
1.6
1.4
1.2
1.0
0.8
0.8
C8H17
H
H
H
H
H
O
5 α-Cholestan-6-one

Fig. 1a

Since the total transformation of vinylnitrosteroidal to (XXXI – XXXIV) to ketones (XXXV – XXXVIII) which is a electron transfer reaction by hydrazine – hydrate (Raney Nickel catalysed) reduction occurred at room temperature with better yields confirmed that hydrazine hydrate – Raney Nickel is better reducing agent ($Ni \rightarrow \frac{1}{2} Ni^{++} + 0.263$ V+ $N_2 H_5 + 3H+$) than zinc – acetic acid combination ($Zn \rightarrow \frac{1}{2} Zn^{++} + 0.761$). Reduction of vinylnitro steroids (XXXI – XXXIV) afforded steroidal ketones (XXXV – XXXVIII) we have used these ketones (XXXV – XXVIII) for 2D – NMR spectral studies.

^{1}H-^{1}H-NMR homonuclear cosy spectrum of 5α-cholestan-6-one (XXXV) (Fig. 1a) :

^{1}H-^{1}H-NMR homonuclear cosy spectrum of 5α-cholestan-6-one (XXX) (Fig. 1a) explains that H-5α at (δ 2.18) as double doublet (Jae = 4.5 Hz, Jaa = 13.5 Hz, axial) was coupled with H-4α (δ 2.04) and H-4β (δ 2.00). H-7β (δ 2.16) appeared as double doublet (Jae = 4.5 Hz and Jgem=13.0 Hz) was coupled with H-7α (δ 2.14) and H-8β (δ 1.86) and H-7α (δ 2.14) was coupled with H-7β (δ 2.16) and H-8β (δ 1.86).

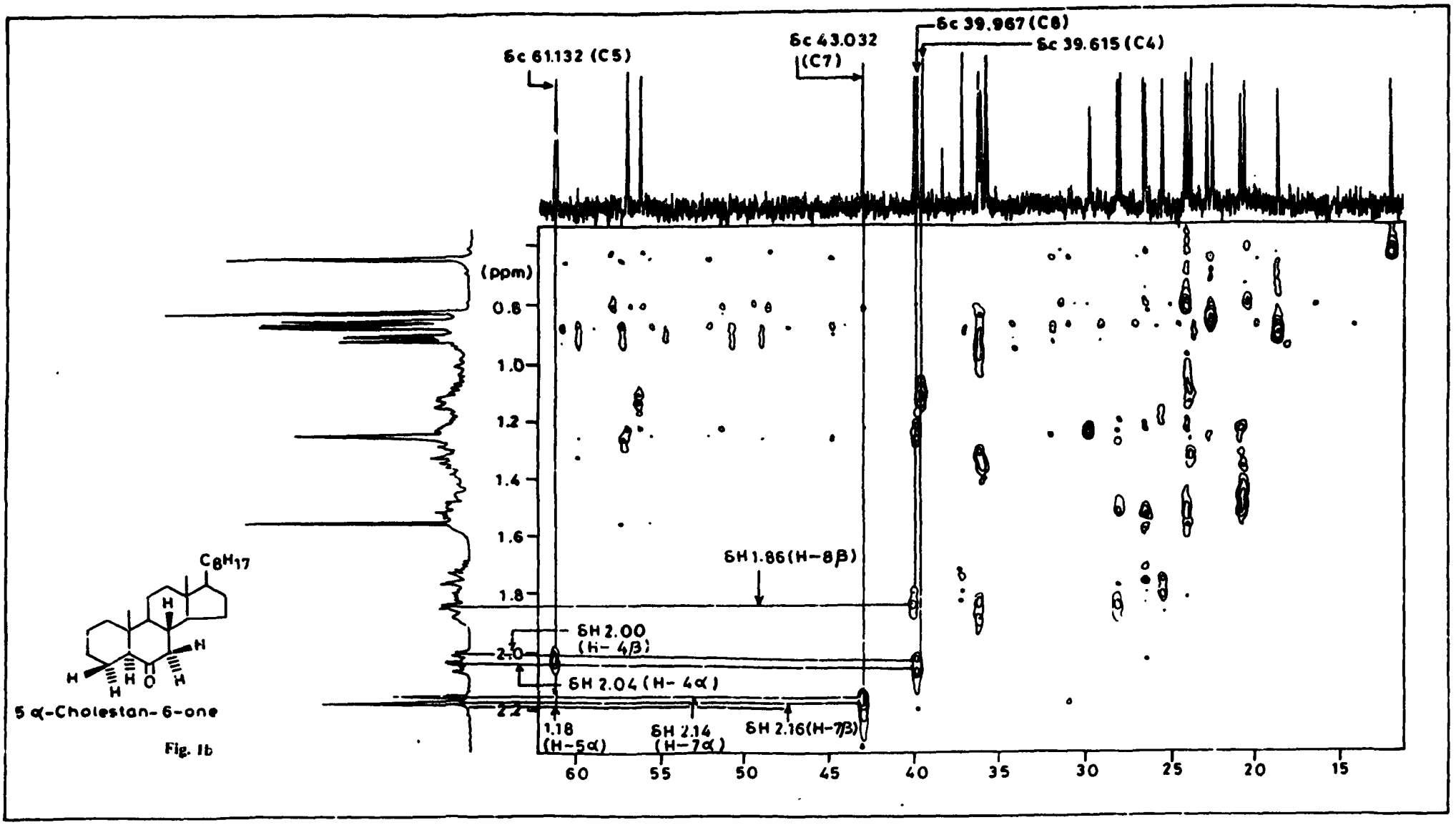
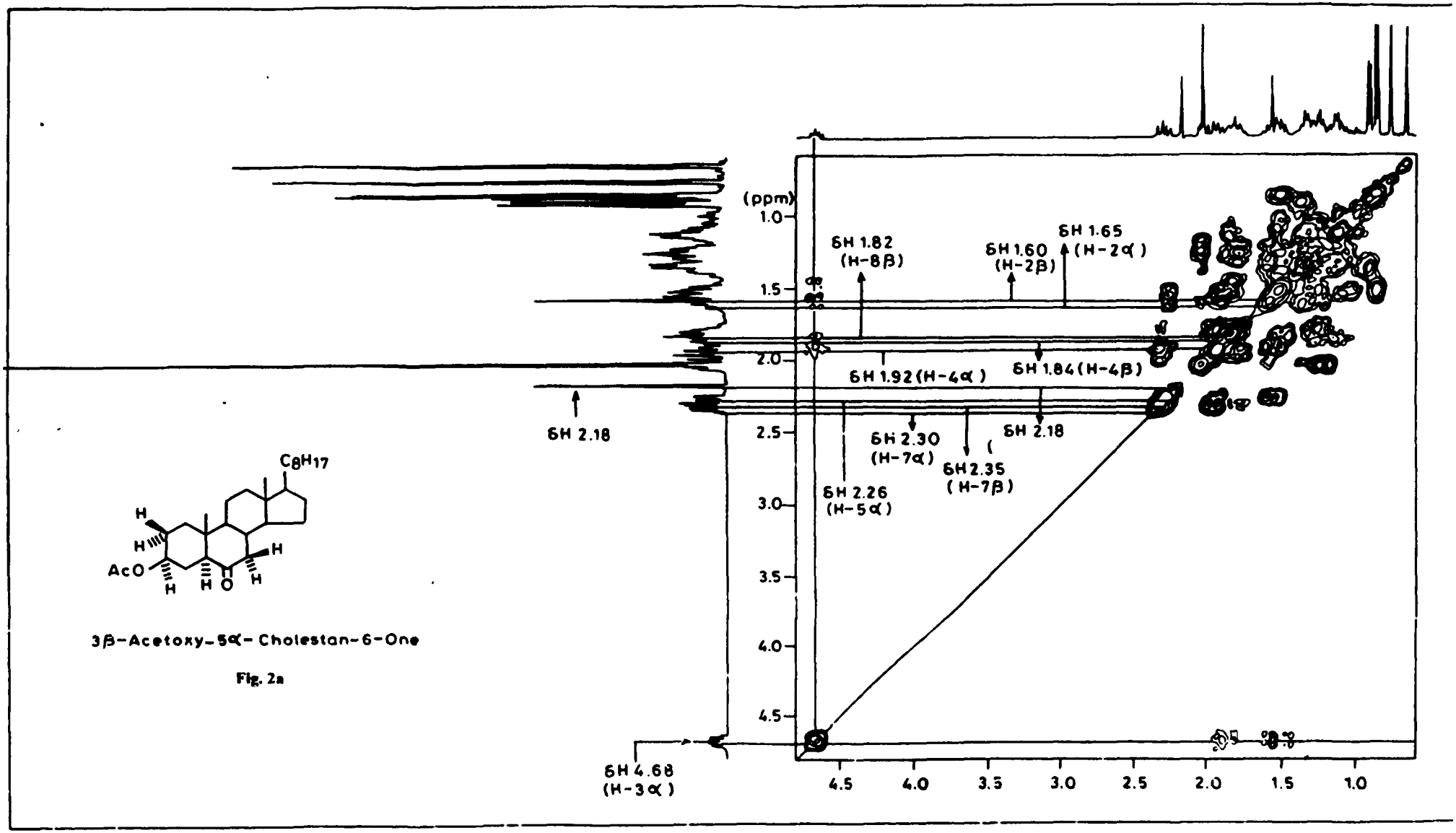

δc 61.132 (C5)
δc 43.032 (C7)
δc 39.967 (C8)
δc 39.615 (C4)
(ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
δH 1.86 (H-8β)
δH 2.00 (H-4β)
δH 2.04 (H-4α)
1.18 (H-5α)
δH 2.14 (H-7α)
δH 2.16 (H-7β)
60
55
50
45
40
35
30
25
20
15
C8H17
H
H
H
H
O
H
5 α-Cholestan-6-one

Fig. 1b



3β-Acetoxy-5α- Cholestan-6-One

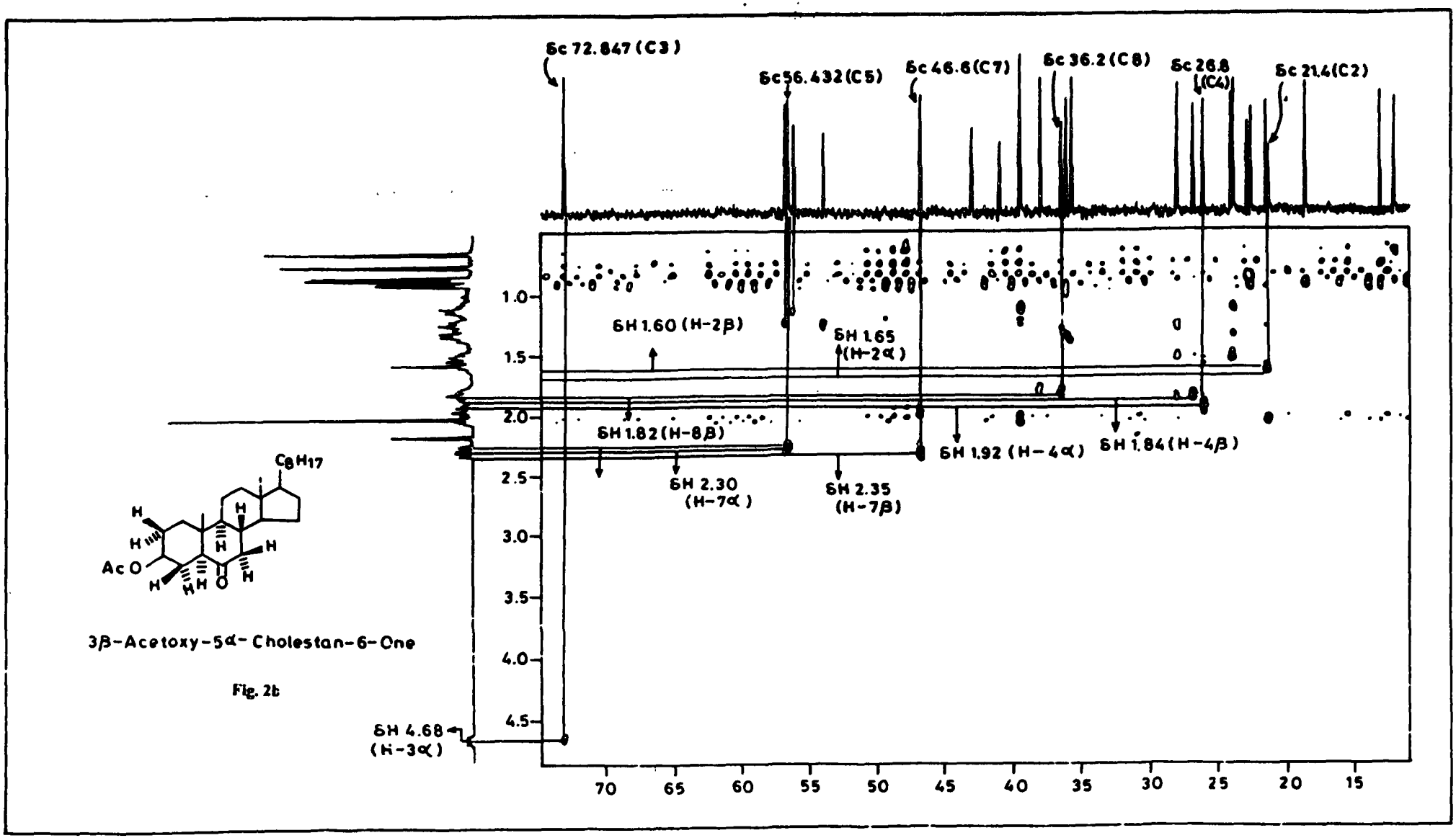
Fig. 2a

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 5α-cholestan-6-one (XXXV) (Fig. 1b) :

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 5α-cholestan-6-one (XXXV)(Fig. 1b) correlates H-4α (δ 2.04), H-4β (δ 2.00) to δ_C 26.656 (C4), H-5α (δ 2.18) to δ_C 61.132 (C5), H-7β (δ 2.16), H-7α (δ 2.15) to δ_C 43.032 and H-8β (δ 1.86) to 39.976 (C8) (one bond correlation).

^{1}H-^{1}H-NMR homonuclear cosy spectrum of 3β-acetoxy-5α-cholestan-6-one (XXXVI) (Fig. 2a) :

The ^{1}H-^{1}H-NMR cosy spectrum of 3β-acetoxy-5α-cholestan-6-one (XXXVI) (Fig. 2a) gave contour on diagonal at δ 4.68 (H - 3α) which is coupled by H - 4α (δ 1.92), H - 4β (δ 1.84), H - 2α (δ 1.65) and H-2β (δ 1.60). H - 5α (δ 2.26) appeared as double doublet (Jae = 4.5 Hz, Jaa = 12 Hz) coupled by H - 4α (δ 1.92) and H - 4β (δ 1.84). A singlet at δ 2.18 in the ^{1}H – ^{1}H cosy spectrum is assigned to protons of acetate methyl. The contour at δ 2.18 on diagonal has no cross over multiplet and therefore acetate methyl protons (δ 2.18) appeared as signalet. The H - 7β appeared as double doublet at δ 2.35 (J = 4.5 Hz and 12 Hz). This proton is coupled by H - 7α (δ 2.30) and H - 8β (δ 1.82).



3β-Acetoxy-5α-Cholestan-6-One

Fig. 2b

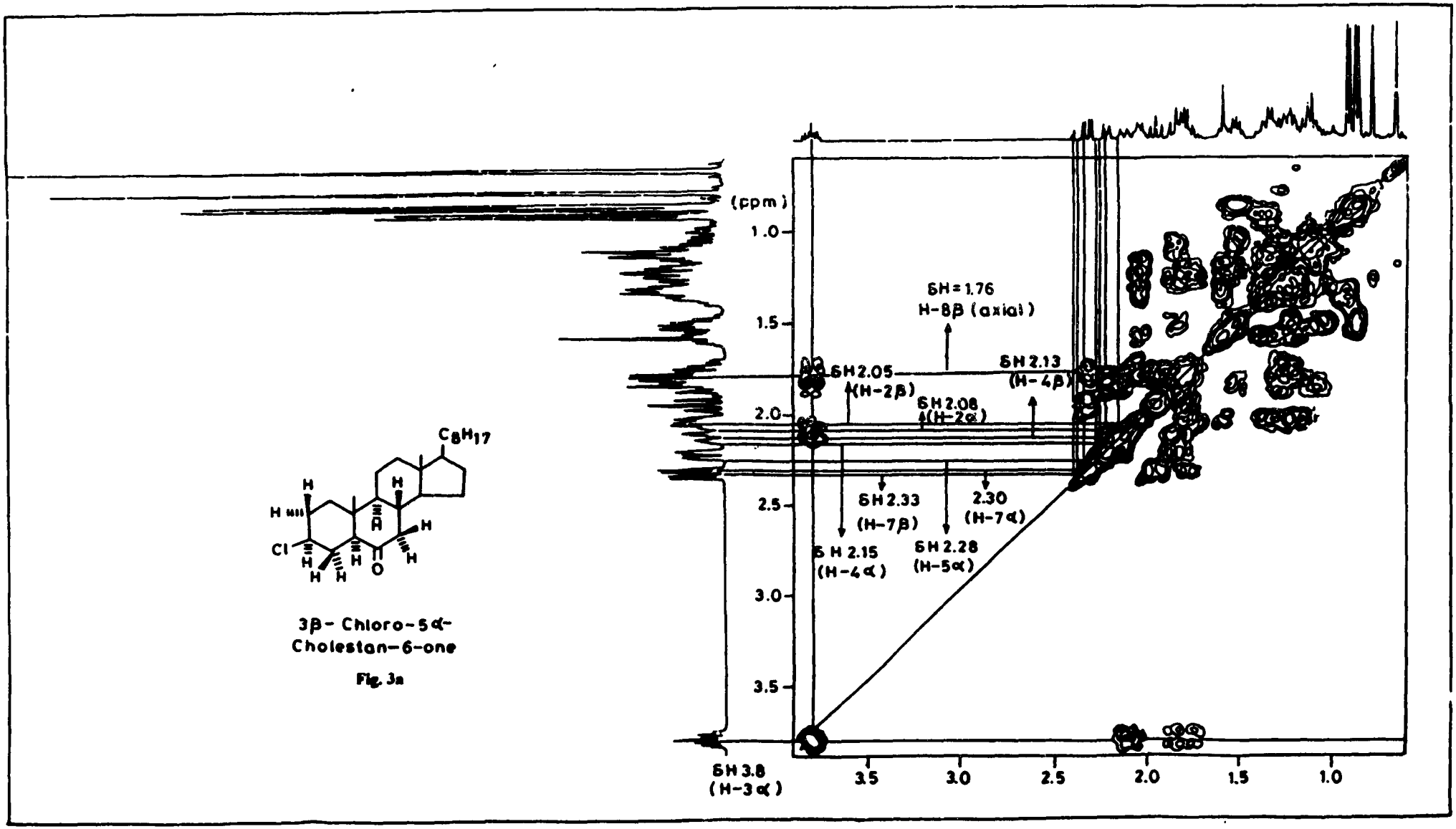

(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
δH = 1.76
H-8β (axial)
δH 2.05
(H-2β)
δH 2.13
(H-4β)
δH 2.08
(H-2α)
δH 2.33
(H-7β)
2.30
(H-7α)
δH 2.15
(H-4α)
δH 2.28
(H-5α)
δH 3.8
(H-3α)
3.5
3.0
2.5
2.0
1.5
1.0
C8H17
H
H
H
Cl
A
O
3β- Chloro-5α-
Cholestan-6-one

Fig. 3a

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β- acetoxy -5α-cholestan-6-one (XXXVI) (Fig. 2b) :

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β-acetoxy-5α-cholestan-6-one (XXXVI) (Fig. 2b) correlates the δ (chemical shift) of protons with their ^{13}C value. δ 4.68 (H - 3α) is correlated to δ 72.847 (C3), δ 2.26 (H-5α) to δ_C 56.432 (C5), δ_C 2.35 (H - 7β) and δ 2.30 (H - 7α) to δ_C 46.6 (C7).

^{1}H–^{1}H–NMR homonuclear cosy spectrum of 3β-chloro-5α-cholestan-6-one (XXXVII) (Fig. 3a) :

^{1}H–^{1}H–NMR homonuclear cosy spectrum of 3β-chloro-5α=-cholestan-6-one (XXXVII) (fig. 3a) gave a multiplet at δ 3.8 (H - 3α) as contour on the diagonal and known as diagonal peak multiplet and on either sides of diagonal cross peak multiplets at δ 2.13 (H - 4β), δ 2.15 (H - 4α), δ 2.05 (H - 2β) and δ 2.08 (H - 2α) coupling with H - 3α which appeared as multiplet. A double doublet at δ 2.33 (Jae = 4.5 Hz and Jgem = 13 Hz) was assigned to H - 7β. This proton being equatorial is coupled by H - 8β (δ 1.76, axial) and H - 7α (δ 2.30, axial) which is correlated by ^{1}H–^{1}H–NMR cosy spectrum. A double

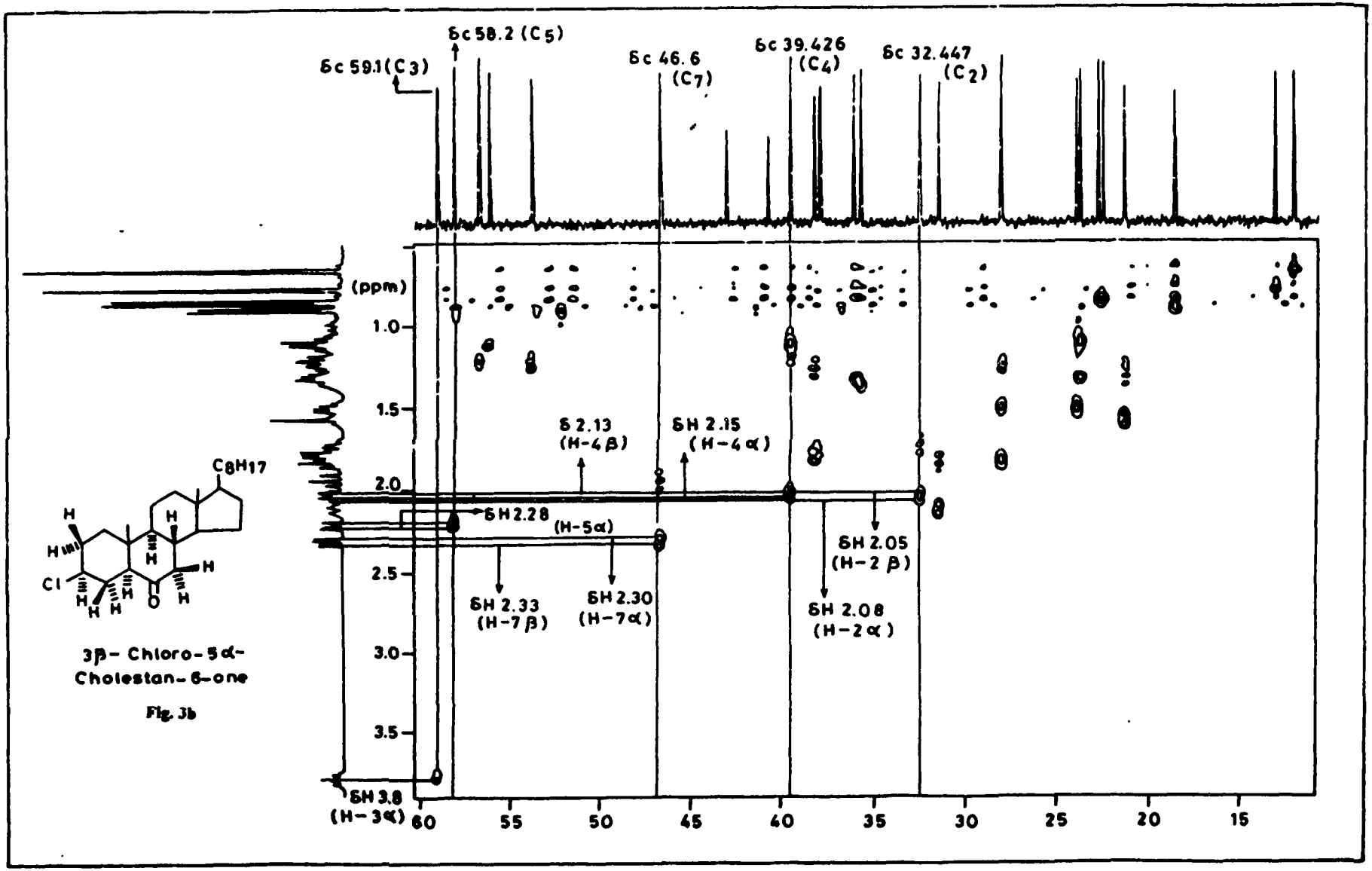
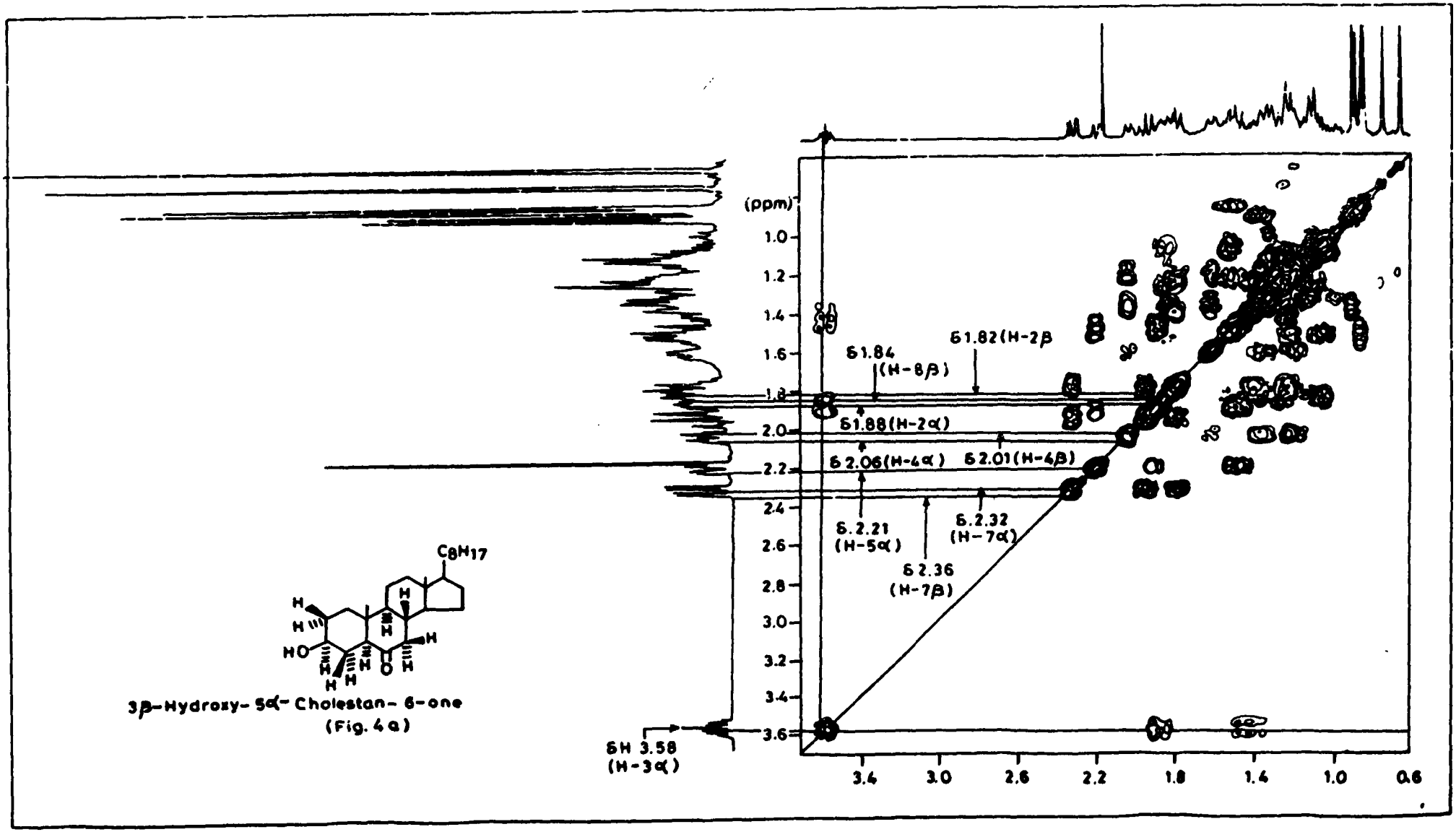

δc 58.2 (C5)
δc 59.1(C3)
δc 46.6 (C7)
δc 39.426 (C4)
δc 32.447 (C2)
(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
δ 2.13 (H-4β)
δH 2.15 (H-4α)
δH 2.28 (H-5α)
δH 2.33 (H-7β)
δH 2.30 (H-7α)
δH 2.05 (H-2β)
δH 2.08 (H-2α)
δH 3.8 (H-3α)
60
55
50
45
40
35
30
25
20
15
C8H17
Cl
O
3β- Chloro-5α-
Cholestan-6-one

Fig. 3b



3β-Hydroxy-5α-Cholestan-6-one
(Fig. 4a)

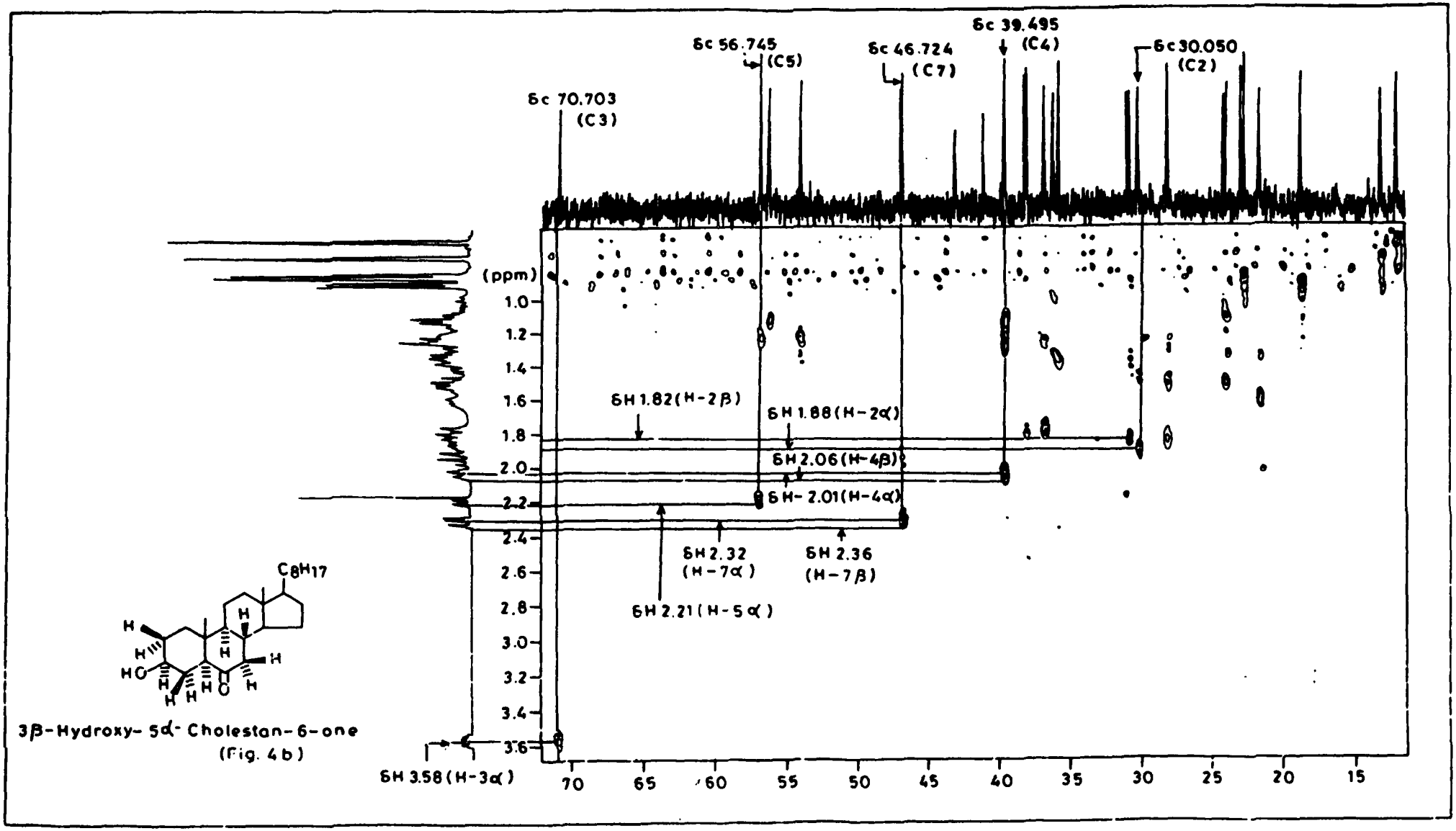
doublet at δ 2.28 (Jae = 4.5 Hz and Jaa = 13 Hz) for one proton is assigned to H - 5α. This proton is coupled by H - 4α (δ 2.15) and H - 4β (δ 2.13).

^{1}H–^{13}C–NMR hetronuclear cosy spectrum of 3β-chloro-5α-cholestan-6-one (XXXVII) (Fig. 3b) :

Chemical shifts (δ) are easily correlated with δ_C; δ 3.8 (H - 3α) is correlated with δ_C 59.1 (C3), δ 2.28 (H - 5α) to δ_C 58.2 (C5), δ 2.33 and δ 2.30 to δ_C 46.532 (C7) and δ 2.05 (H - 2β) and δ 2.08 (H - 2α) are finally correlated to δ_C 32.447 (C2).

^{1}H-^{1}H-NMR homonuclear cosy spectrum of 3β-hydroxy-5α-cholestan-6-one (XXXVIII) (Fig. 4a) :

^{1}H-^{1}H-NMR homonuclear cosy spectrum of (XXXVIII) (Fig. 4a) has made it clear that H-3α gives peak at δ 3.58 (W½ = 18 Hz) as multiplet was coupled by H-4α (δ 2.06), H-4β (δ 2.01), H-2α (δ 1.88) and H-2β (δ 1.82). H-5α at (δ 2.21) was coupled by H-4α (δ 2.06) and H-4β (δ 2.01). H-7β (δ 2.36) was coupled by H-7α (δ 2.32) and H-8β (δ 1.84). Coupling was observed between H -7α (δ 2.32) and H-7β (δ 2.36), H-8β (δ 1.84).



3β-Hydroxy-5α-Cholestan-6-one
(Fig. 4b)

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β-hydroxy-5α-cholestan-6-one (XXXVIII-b) (Fig. 4b) :

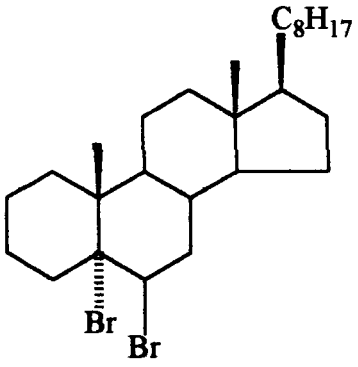
^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β-hydroxy-5α-cholestan-6-one (XXXVIII) (Fig. 4b) has shown that H-2α (δ 1.88), H-2β (δ 1.82) was correlated to δ_C 30.050 (C2), H-3α (δ 3.58) to δ_C 70.703 (C3), H-4α (δ 2.06), H-4β (δ 2.01) to δ_C 39.495 (C4), H-5α (δ 2.21) to δ_C 56.745 (C5), H-7β at (δ 2.36), H-7α (δ 2.32) to δ_C 46.724 (C7). The H-8β was correlated to δ_C 37.909 (C8).

CHAPTER-THREE

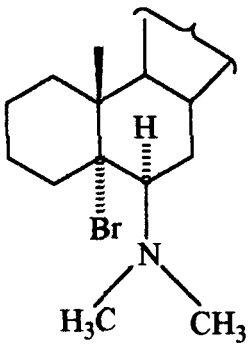
Reactions of Dibromosteroids with Organic Bases.

The manifold physiological properties associated with a variety of compounds containing hetero atoms with useful therapeutic values prompted us to carry out extensive research in this field. Steroids a class of biologically active compounds were modified to a variety of oxygen and nitrogen containing derivatives, playing a vital role in the era of medicine and drugs and synthetic organic chemistry. These compounds were found to possess dermatological, opthalmic, antiulcer, immunoassay and CNS depressant activities in association with other physiological activities. The present work describes the reaction of 5, 6β-dibromo-5α-steroids (XXXIX) and 3 keto-5, 6β-dibromo-5α-steroids (XL) with dimethylamine, succinimide and morpholine. The reactions of 5, 6β-dibromo-5α-cholestane (XXXIX) in benzene with dimethylamine, succinimide and morpholine at room temperature for half on hour, afforded 5-bromo-6β-dimethylamino-5α-choleatne (XLI), 5-bromo-6β-amino-5α-cholestane (XLII) and 5 bromo-6β-morpholino-5α-cholestane (XLIII) respectively. When 5, 6β-dibromo-5α-cholestan-3-one (XL) was treated with morpholine and 2-aminopyrimidine under identical reaction conditions gave 6β-morpholinocholest-4-en-3-one (XLIV) and 6β-aminopyrimidinocholest-4-en-3-one (XLV). The structure of

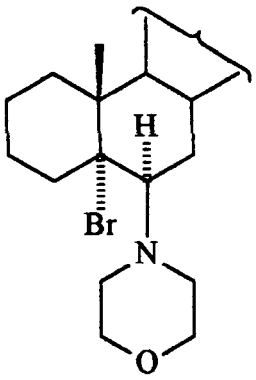
these compounds was established on the basis of analytical and spectral (IR, ^{1}H-NMR, ^{13}C-NMR and Mass) evidences.



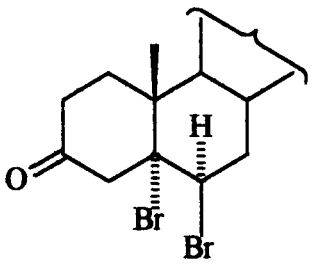
(XXXIX)



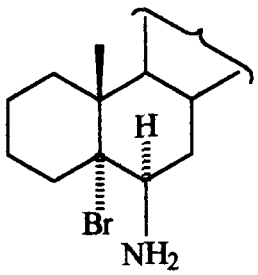
(XLI)



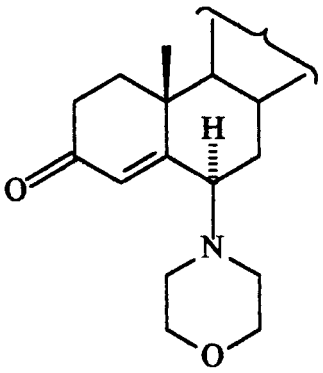
(XLIII)



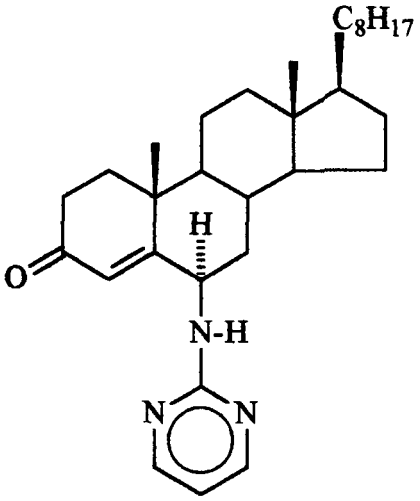
(XL)



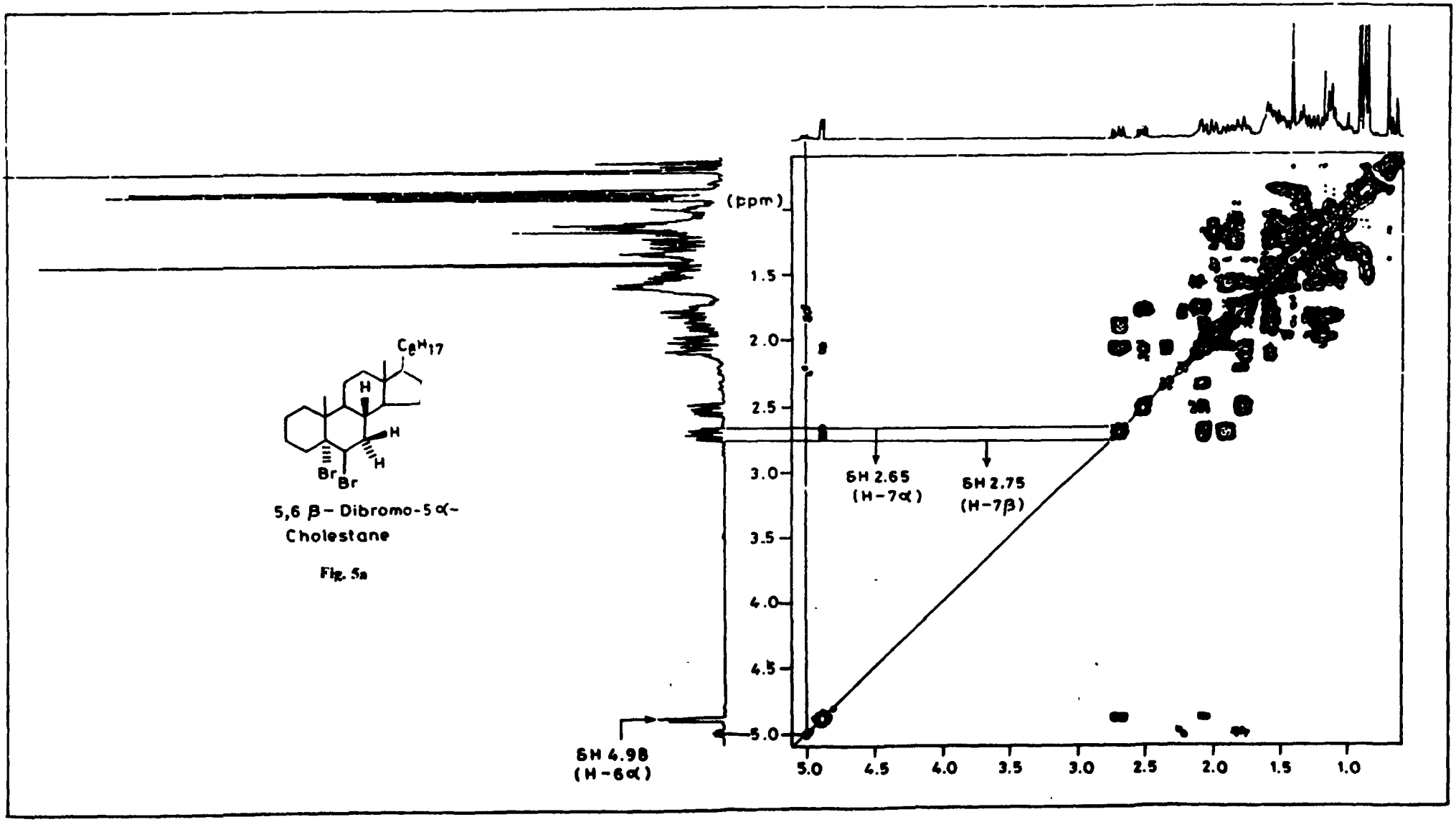
(XLII)



(XLIV)

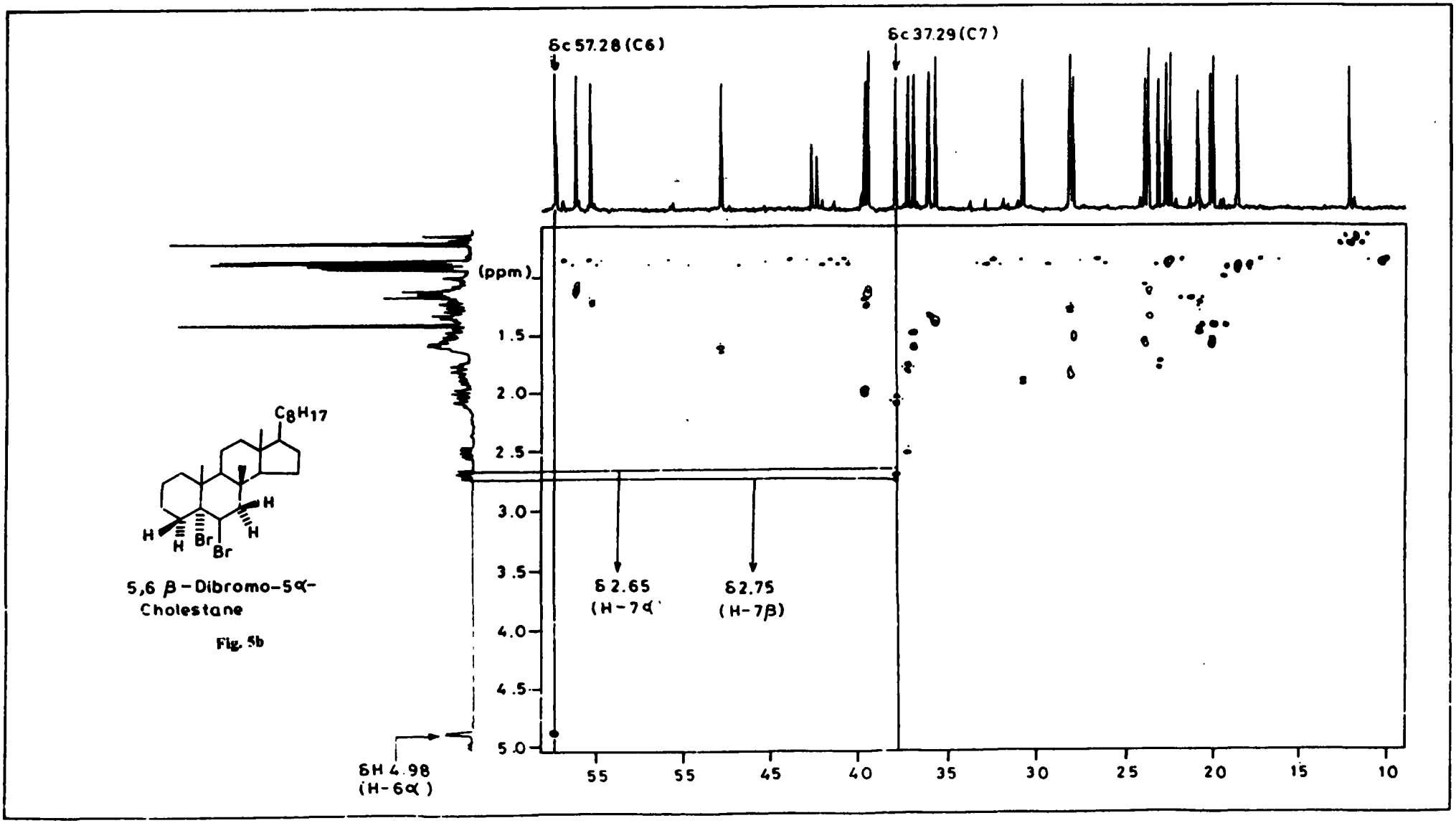


(XLV)



5,6 β – Dibromo-5α-
Cholestane

Fig. 5a

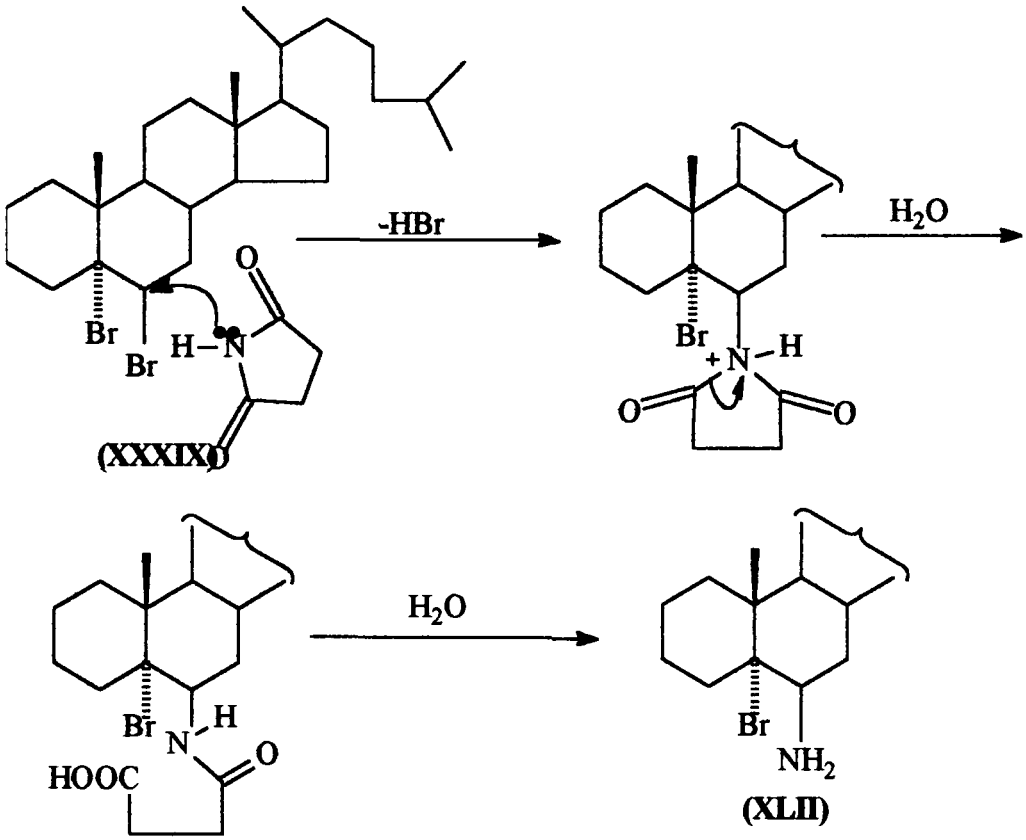


5,6 β-Dibromo-5α-Cholestane

Fig. 5b

The spectral studies of 5, 6β-dibromo-5α-cholestane (XXXIX) the starting compound was done in detail with special reference to 2D-NMR spectroscopy to support the orientation of bromine atoms at C5 (α-oriented, axial) and C6 (β-oriented, axial) and its purity. ^{1}H-^{1}H-NMR homonuclear and ^{13}C-NMR hetronuclear cosy spectrum (Fig. 5a,b) correlated H-6α (δ 4.98) which appeared as double doublet coupled by H-7α (Jea = 4 Hz) and H-7β (Jee = 2 Hz) to δ_C57.28 and H-7α (δ 2.65) and δ H-7β (δ 2.75) to δ_C 37.29 (C7).

Formation of products (XLI, XLIII, XLIV and XLV) involved simple substitution reaction where bromine is replaced by dimethylamine, morpholine and 2-aminopyrimidine, but the formation of 5-bromo-6β-amino-5α-cholestane (XLII) obtained by the reaction of 5, 6β-dibromo-5α-cholestane (XXXIX) with succinimide in benzene can be explained by following mechanism

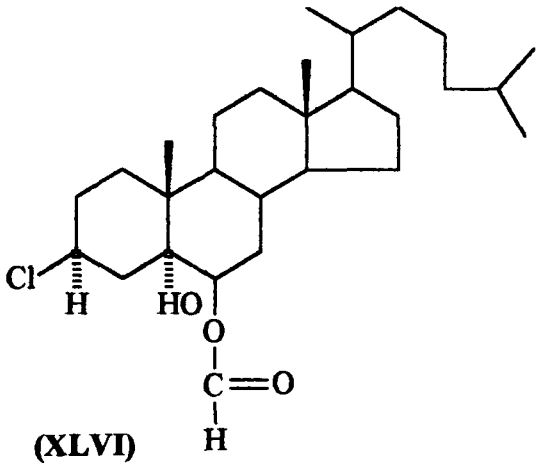

-HBr
H_2O
Br
Br
H–N
O
(XXXIX)
Br
+N–H
O
O
Br
N
H
HOOC
O
H_2O
Br
NH_2
(XLII)

CHAPTER-FOUR

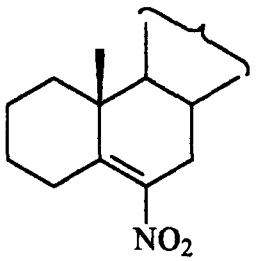
Applications of X-rays In Structure Elucidation of Steroids :

In recent years X-rays methods have been increasingly used qualitative and quantitative analysis as well as for fundamental studies of the properties and structure of various class of both organic and inorganic compounds. X-ray diffraction is the only convenient and hence widely used physical procedure for the complete determination of molecular structure.

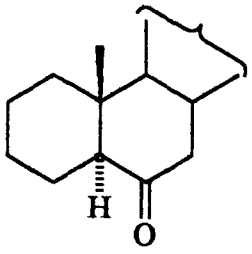
Previous work from these laboratories described crystallographic studies of 3β-chloro-6β-formyloxy-5α-cholestan-5-ol (XLVI), 6-nitrocholest-5-ene (XLVII), 5α-cholestan-6-one (XLVIII) and 3β-chloro-6-nitrocholest-5-ene (XLIX).



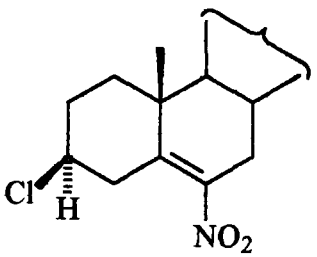
(XLVI)



(XLVII)

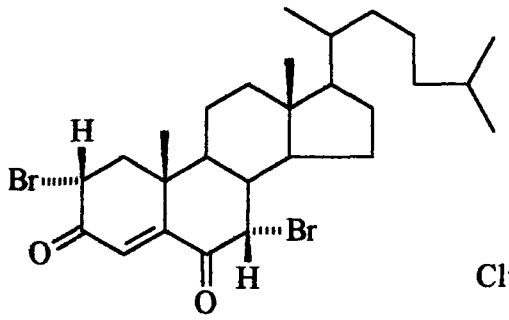


(XLVIII)

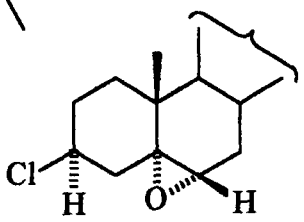


(XLIX)

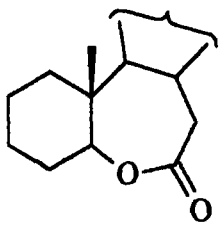
In continuation, as part of X – ray studies of steroids we have taken few steroidal compounds of cholestane series synthesized in our laboratory such as 2α, 7α-dibromocholest-4-ene-3, 6-dione (L), 3β-chloro-5, 6α-epoxy-5α-cholestane (LI), 6-oxa-B-homo-5α-cholestan-7-one (LII) and 5α-cholestane-3, 6-dione (LIII). In each case different parameters and well as bond length bond angle of various bonds present in each molecule, obtained by X- ray analysis are given. Conformational changes occurred during the formation of these compounds (L – LIII) are also studied.



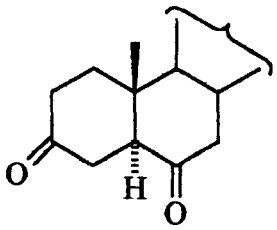
(L)



(LI)



(LII)



(LIII)

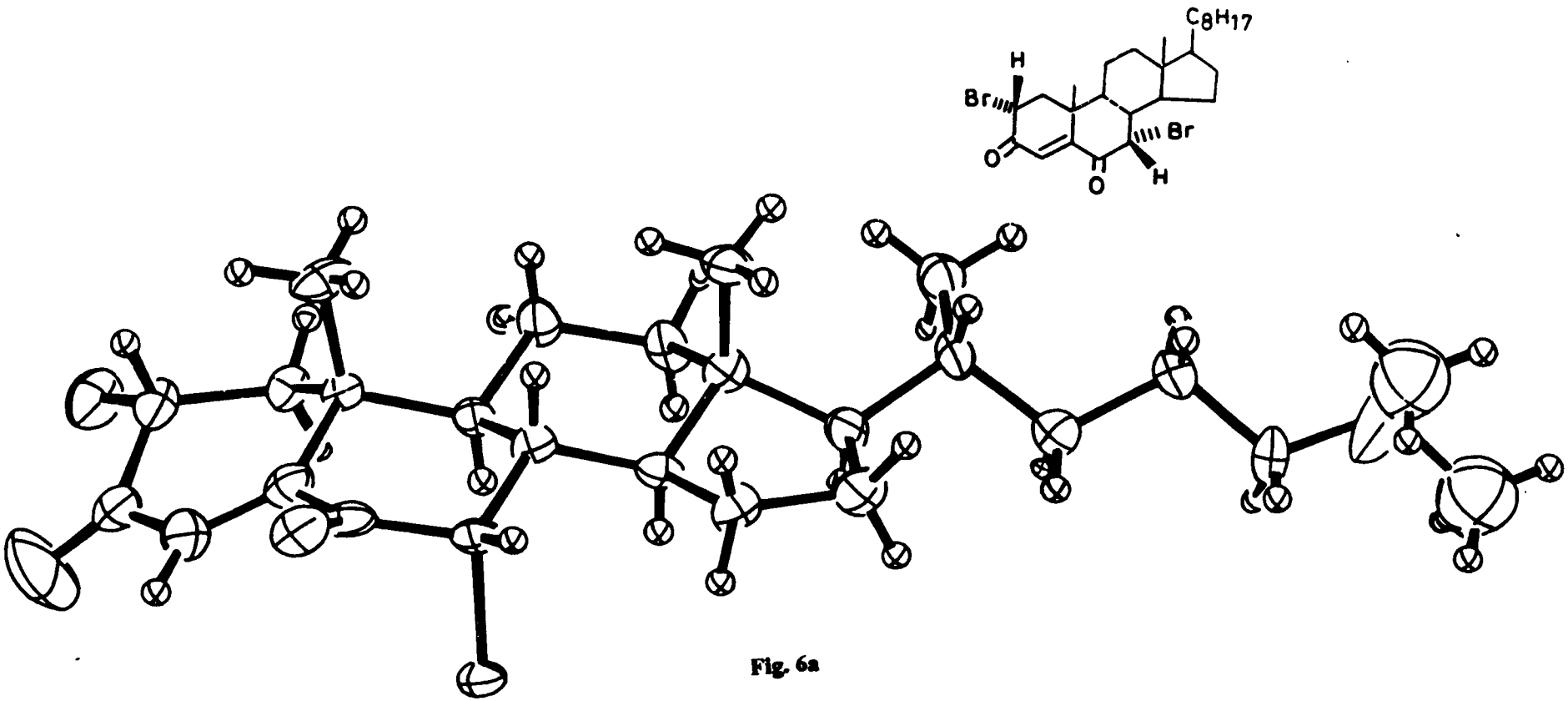


Fig. 6a

General view of the molecule

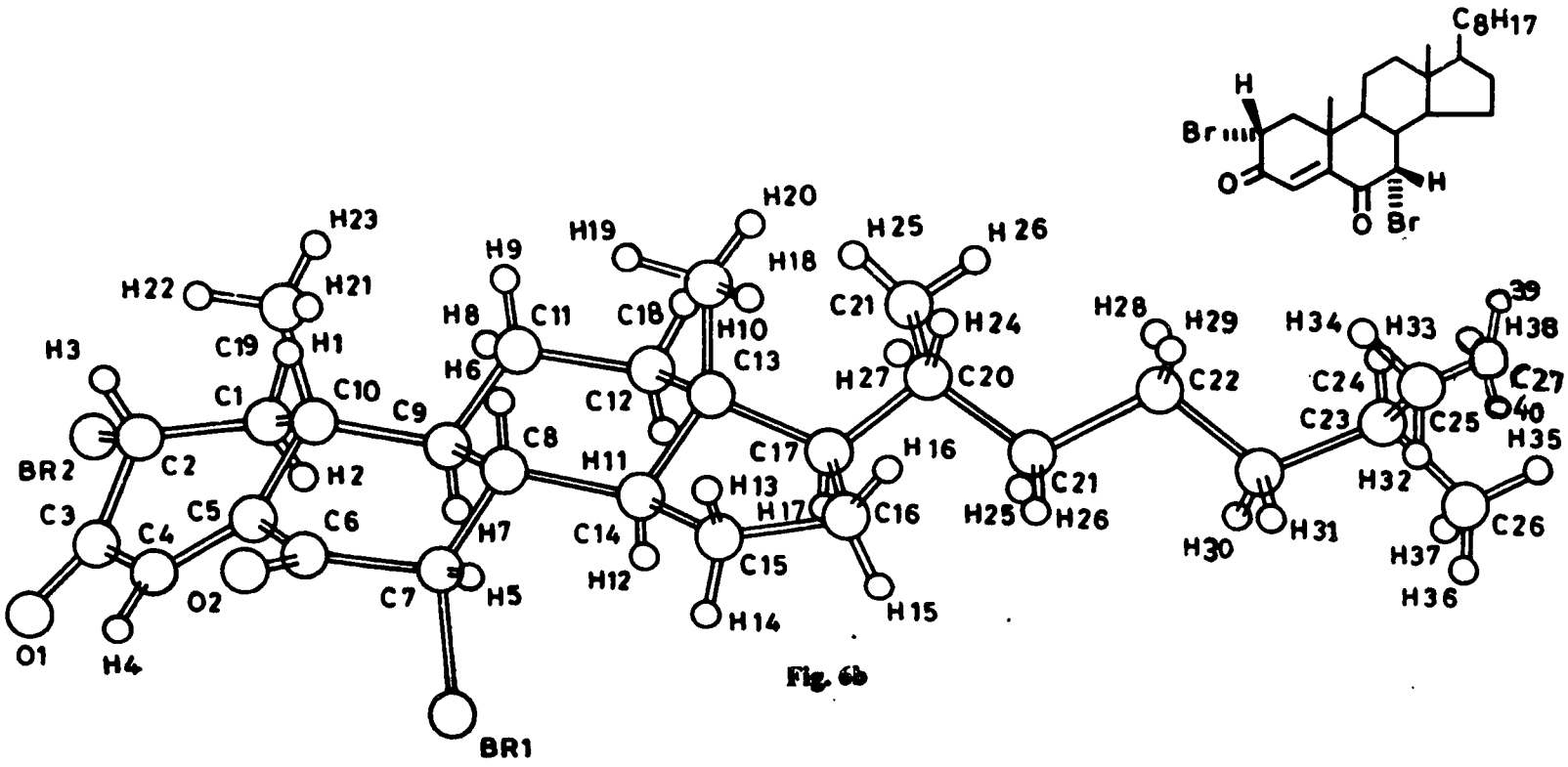


Fig. 6b

Plot showing atomic arrangement and numbering

X-Ray analysis of 2α, 7α-dibromocholest-4-ene-3, 6-dione (L) (Fig. 6a, b) :

The X-ray analysis of 2α, 7α-dibromo cholest-4-ene-3, 6-dione (L) ($C_{27}H_{40}O_2Br_2$, molecular weight = 556.62) was done with crystal morphology results: colourless, prism, crystal dimensions (mm) 0.50 x 0.50 x 0.50, crystal system; monoclinic, lattice dimensions; a = 11.585 (2), b = 7.648 (2), c = 15.323 (1) A°, β = 93.803 (9) A°; volume, 1354.6 (4) $A°^3$, space group, $P2_1$; Z = 2 (two molecules per unit cell), density; 1.364 g/cm^3; radiation, Cukα λ = 1.54178 A°; temperature, 23 °C, structure solutions; direct methods.

In compound 2α, 7α-dibromocholest-4-ene-3, 6-dione (L) (Fig. 6a, b) C2 to C7 carbon atoms with tendency to be in same plane due to Sp^2-nature of the carbons involved causing significant change in the conformation of rings A and B. It is interesting to note that bromine attached to C2 (being equatorial) is lying very close to the plane of the carbonyl group while bromine at C7 (being axial) is moving away from carbonyl plane.

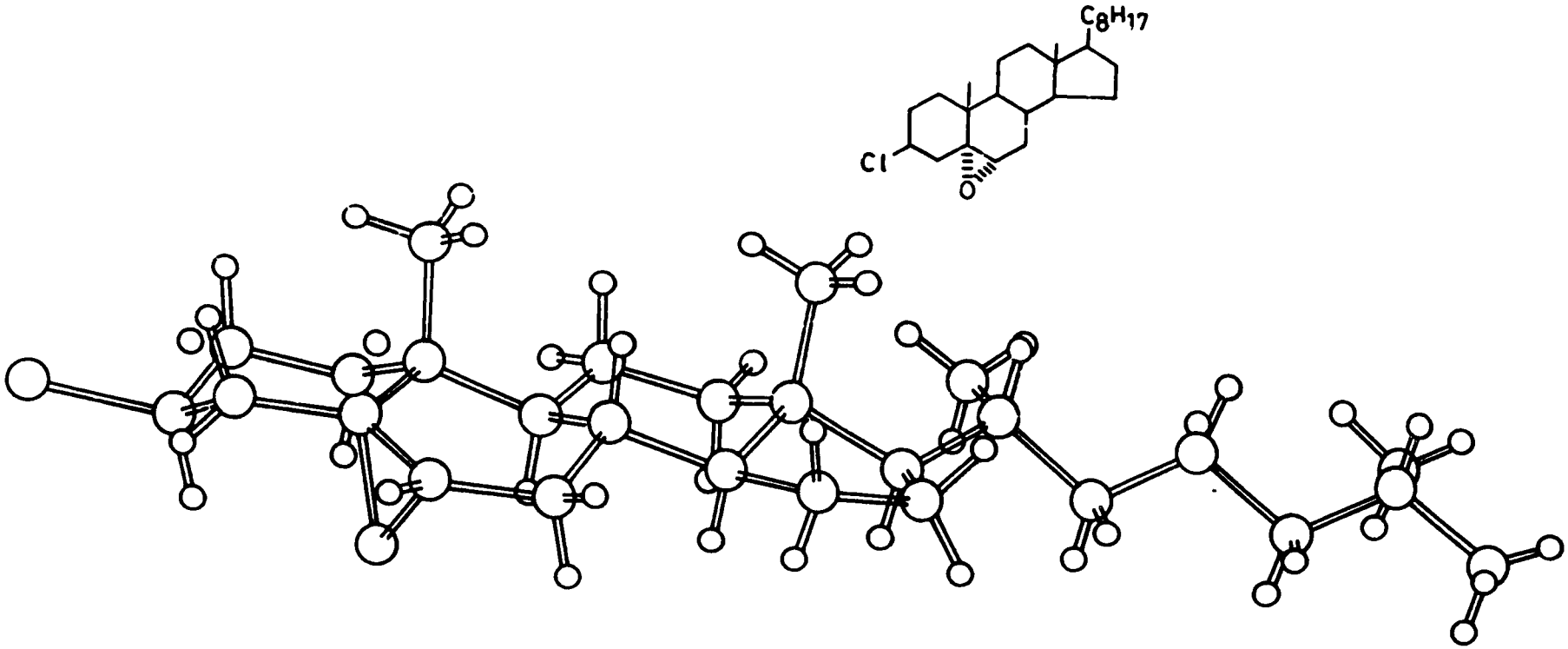


Fig. 7a

General view of the molecule

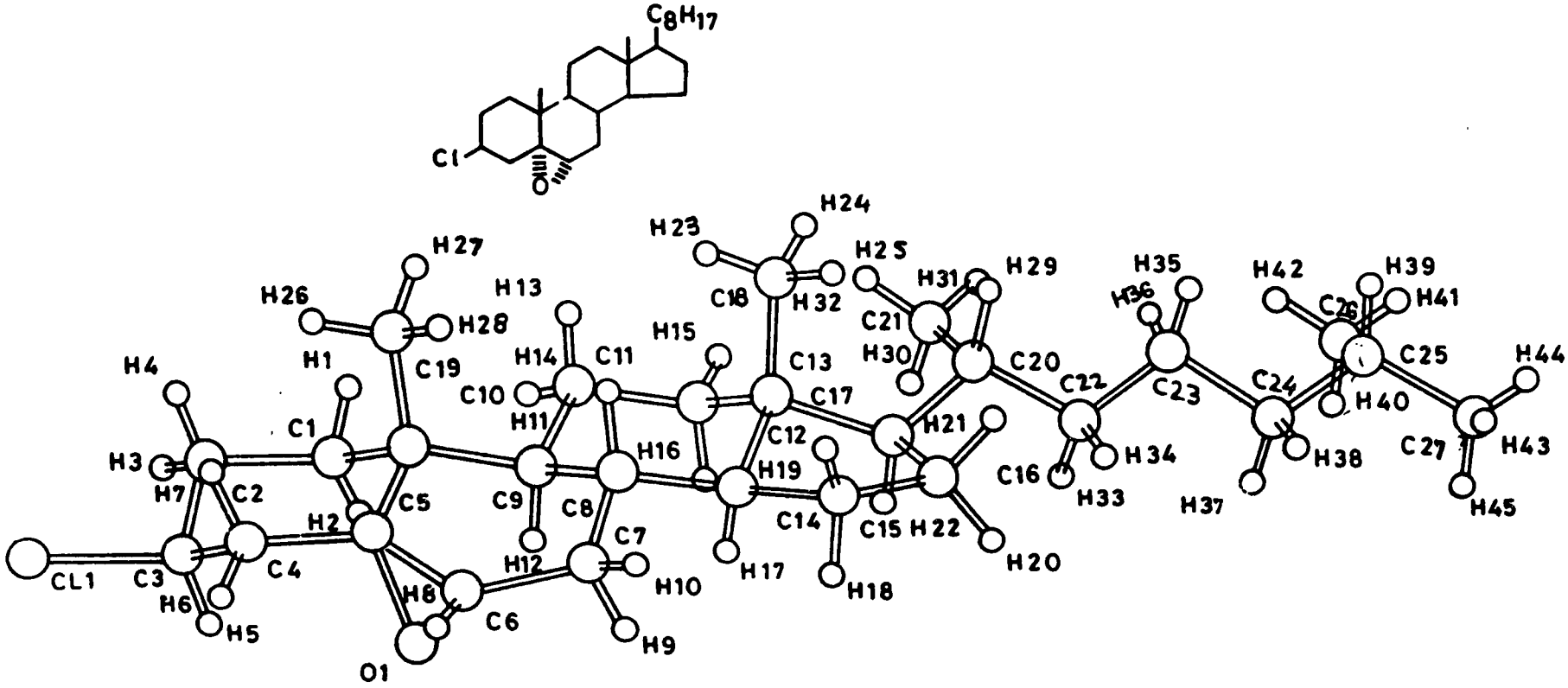


Fig. 7b

Plot showing atomic arrangement and numbering

X-Ray analysis of 3β-chloro-5, 6α-epoxy-5α-cholestane (LI) (Fig. 7a, b) :

X-Ray analysis of 3β-chloro-5, 6α-epoxy-5α-cholestane (LI) was done with the aim to know the conformational changes occurring in the molecule during the formation of epoxide from 3β-chlorocholest-5-ene on treatment with m-chloroperbenzoic acid. It has been found that the conformational changes in rind A and B particularly due to the formation of epoxide ring has occurred. The various parameters obtained during the X-ray crystallographic study : molecular formula; $C_{27}H_{45}ClO$, molecular weight; 421.10, crystal morphology; colourless, plate, crystal dimension (mm); 0.20 x 0.40 x 0.20, crystal system; orthorhombic, lattice parameters; a = 22.80 (4), b = 7.671 (3) 28.657 (5) A°, y = 5012 (2) $A°^3$, space group; $P2_12_12_1$, Z = 8, density 1.116 g/cm^3, radiation; wkα; 1.54178 A°, temperature; 23°C, structure solution; direct methods.

Due to the formation of oxirane ring bond angles around C5 and C6 in compound 3β-chloro-5, 6α-epoxy-5α-cholestane (LI) (Fig. 7a, b) under go changes which are causing strain and geometrical deformation in rings A and B.

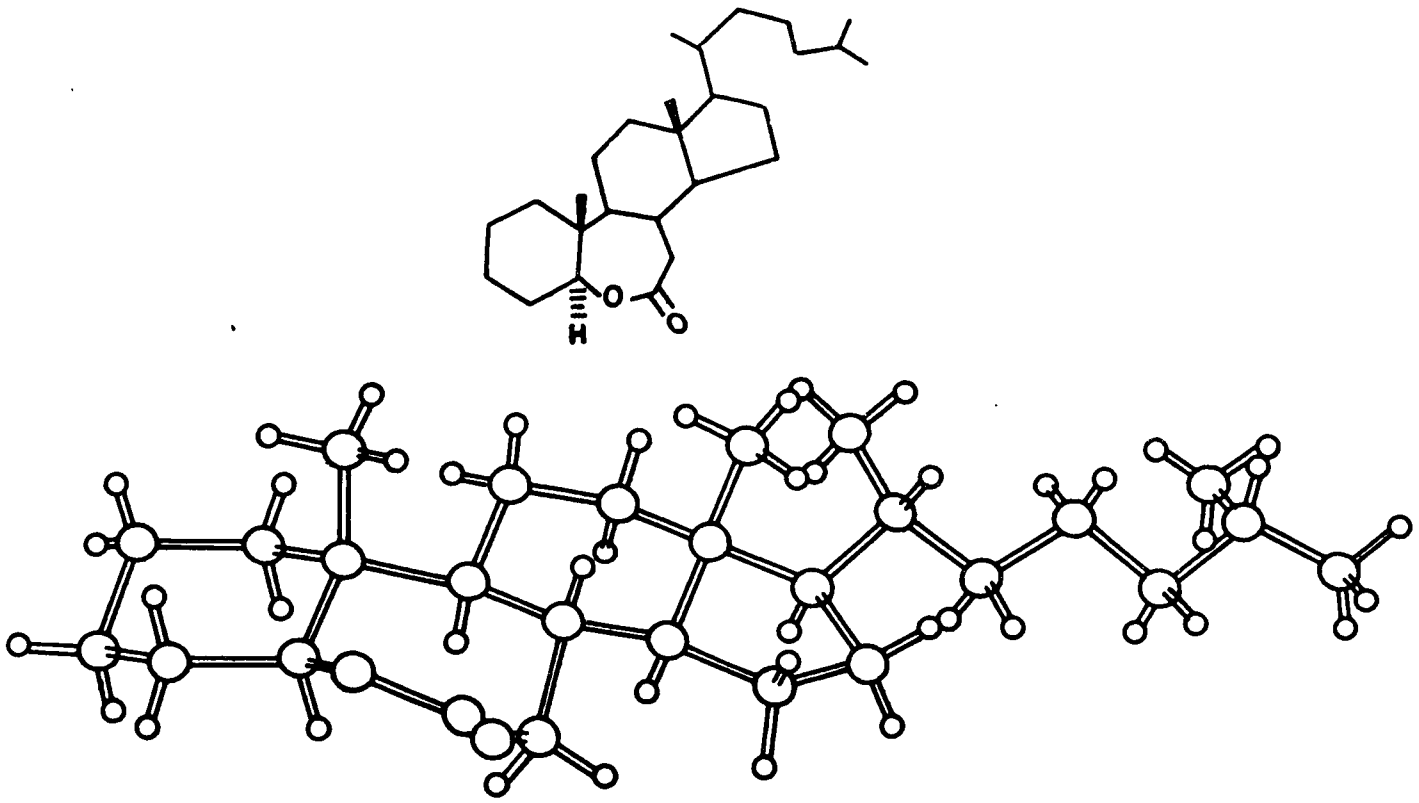


Fig. 8a

General view of the molecule

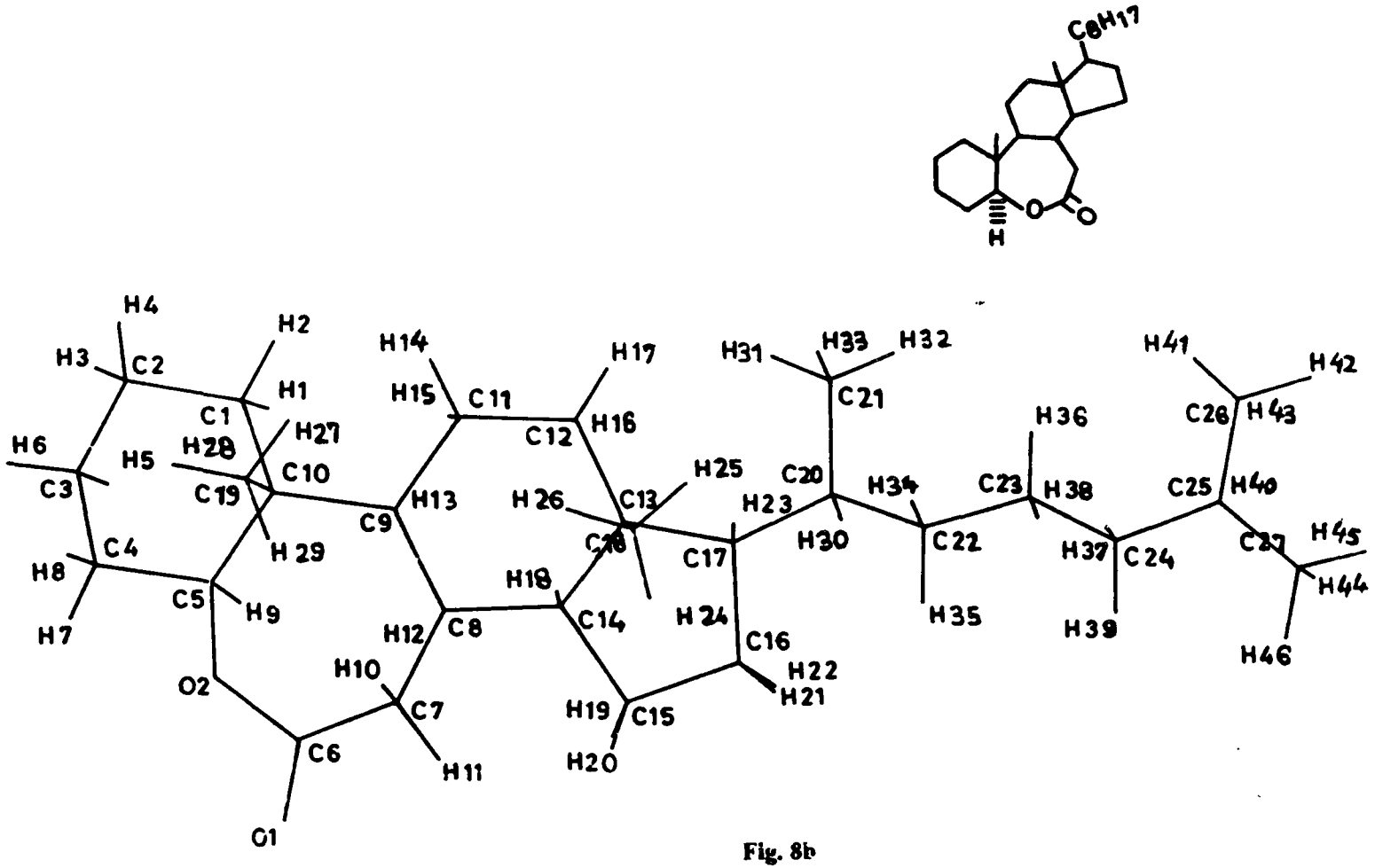


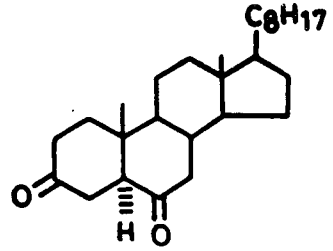
Fig. 8b

Plot showing atomic arrangement and numbering

The X-Ray crystal study of 6-oxa-B-homo-5α-cholestan-7-one (LI) (Fig. 8a, b) :

During the preparation of oxasteroids, when 5α-cholestan-6-one was treated with perbenzoic acid in chloroform (p-toluenesulphonic acid as catalyst) 6-oxa-B-homo-5α-cholestan-7-one (LII) was obtained which was characterized by (IR, ^{1}H-NMR, Mass). The assigned structure was further supported by X-ray crystallography. The result obtained were : molecular formula; $C_{27}H_{46}O_2$, molecular weight; 402.66, crystal morphology; colourless plate; crystal dimension; 0.10x0.20x0.10, crystal system; monoclinic, lattice parameters; a = 5.971 (2), b = 11.043 (1), c = 19.243 (1) A°, space group; $P2_1$, Z = 2 (two molecules per unit cell), density; 1.062 g/cm^3, radiation; Wkα(λ=1.54178 A°), temperature; 23° C structure solution; Paterson method.

Formation of 6-oxa-B-homo-5α-cholestan-7-one (LII) (Fig. 8a, b) from 5α-cholestan-6-one via Baeyer Villiger oxidation involves inlargement of B ring from six to seven membered and lactone moiety having Sp^2-hybridized carbon atom with tendency to have planar geometry causes deformation in seven membered B-ring. It is pertinent to mention that ring C also suffers slight conformational change.



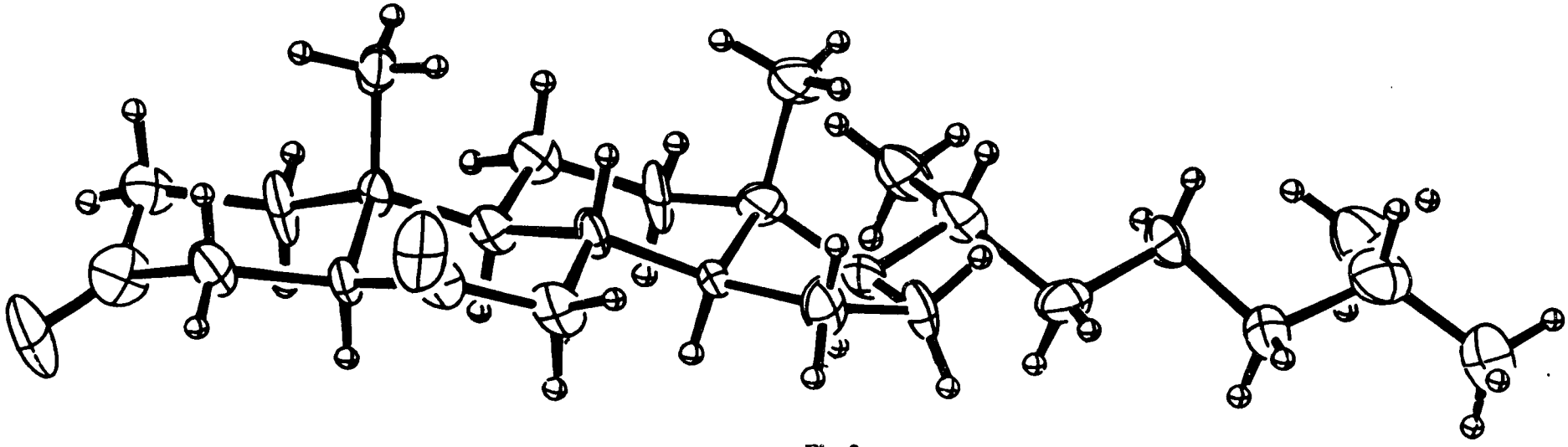


Fig. 9a

General view of the molecule

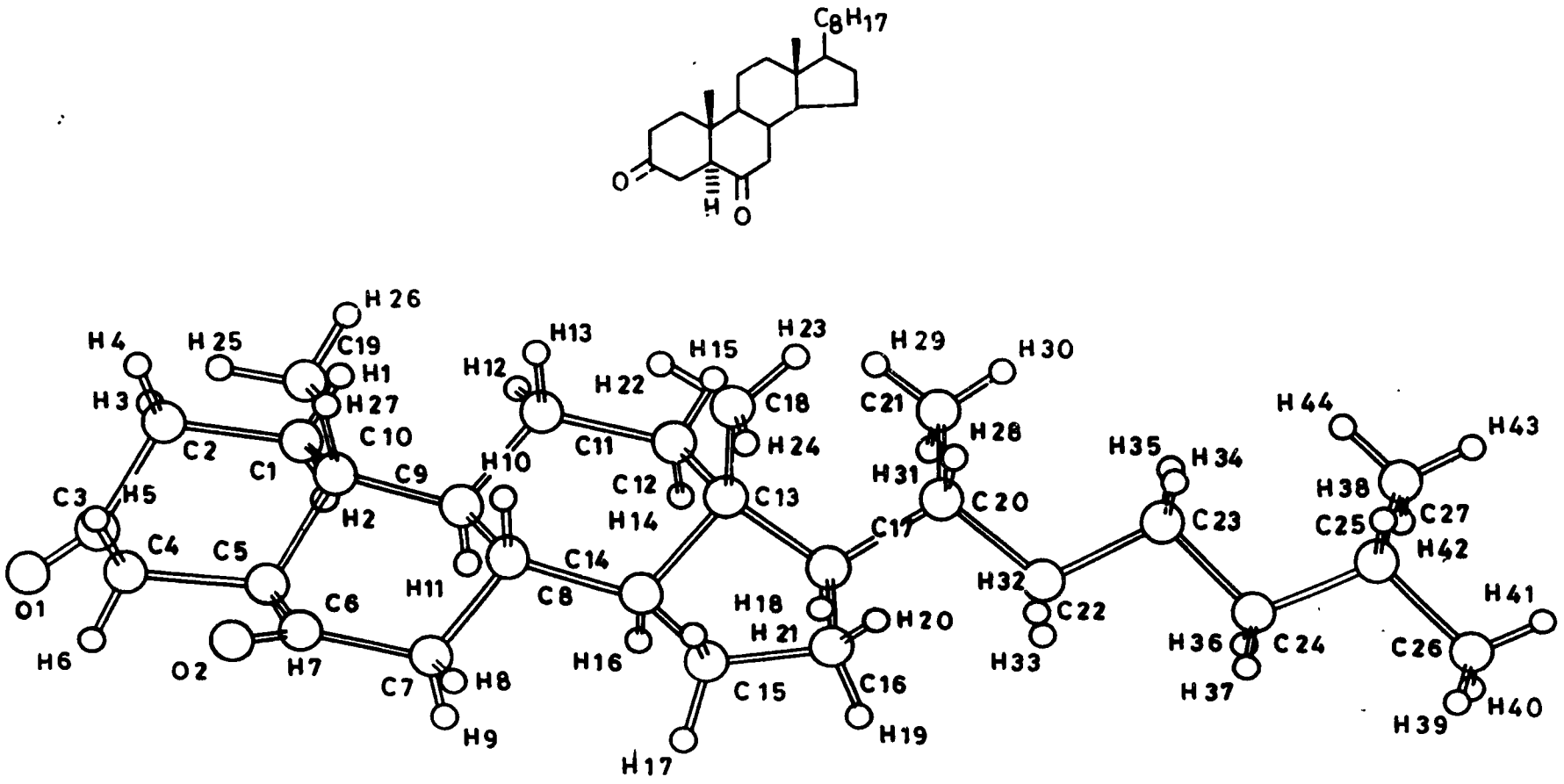


Fig. 9b

Plot showing atomic arrangement and numbering

The X-Ray analysis of 5α-Cholestane-3,6-dione(LIII)(Fig. 9a,b):

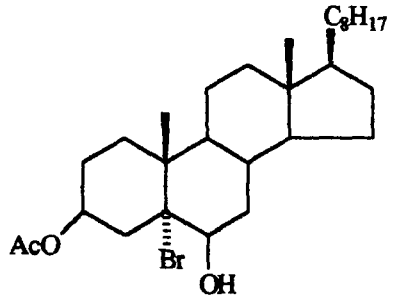
The most interesting compound involved in synthesis of variety of steroidal compound is 5α-cholestane-3, 6-dione (LIII). This compound was prepared, characterized and its detail study of X-ray crystallography was done. The results are : molecular formula, $C_{27}H_{44}O_2$; molecular weight, 400; crystal morphology; colourless plate; crystal dimensions (mm); 0.20x0.50x0.30, crystal system; monoclinic, lattice parameters; a = 8.216 (3), b = 7.616 (2), c = 19.706 (3) A°, β=92.86 (2), volumes; 1231.6 (5) $A°^3$, space group; $P2_1$, (≠4), Z value; 2 (two molecules per unit cell), radiation ; Cu Kα = 1.5417 A°, temperature; 23° C, structure solution; direct method.

In the structure of 5α-cholestane-3, 6-dione (LIII) (Fig. 9a, b) C3 and C6 are Sp^2 – hybridized carbon atoms. Due to this the respective portions (C2, C3, C4 and C5, C6 C7) acquiring planority cause definite conformational deformity in both rings A and B, their conformation became pseudo chair form.

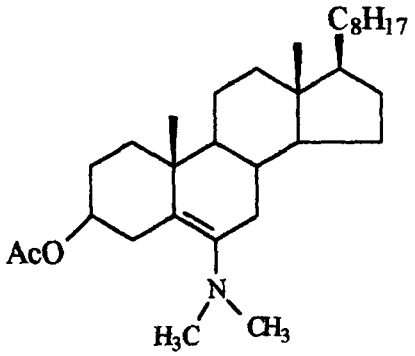
CHAPTER-FIVE

Neurotoxicological Effects of Steroidal Compounds on Lipid Metabolism in Different Regions of Rat Brain :

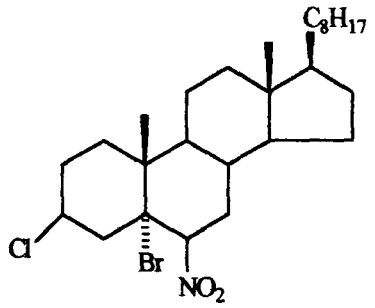
Steroidal compounds 3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane (LIV), 3β-acetoxy-6-dimethylamino cholest-5-ene (LV), 3β-chloro-5-bromo-6β-nitro-5α-cholestane (LVI) and 6β-aminopyrimidino cholest-4-en-3-one (LVII) were designated as A, B, C and D respectively. The present study was under taken to evaluate the neurotoxic effects of these steroidal compounds on lipid metabolism.



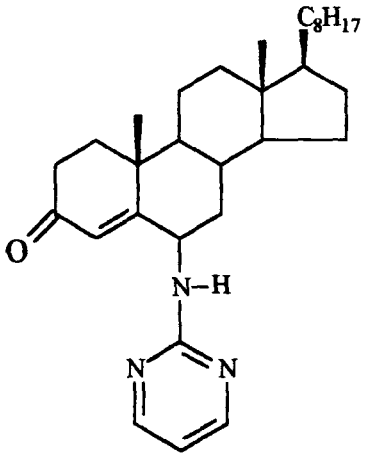
3β-Acetoxy-5-bromo-6β-hydroxy-5α-cholestane (LIV) (A)



3β-Acetoxy-6-dimethylamino cholest-5-ene (LV) (B)



3β-Chloro-5-bromo-6β-nitro-5α-cholestane (LVI) (C)

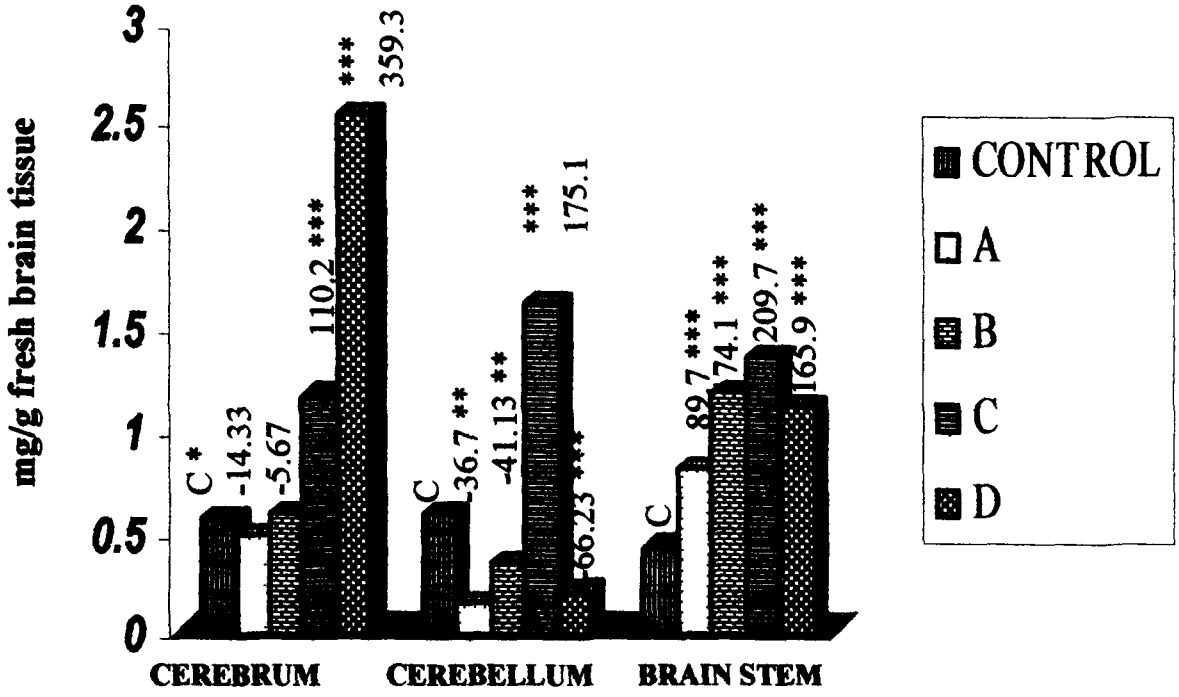


6β-Aminopyrimidino-cholest-4 en-3-one (LVII) (D)

This study shows the effects of four steroidal derivatives (3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane(LIV) (A); 3β-acetoxy-6-dimethylamino-cholest-5-ene (LV) (B); 3β-chloro-5-bromo-6β-nitro-5α-cholestane (LVI) (C); 6β-aminopyrimidino-cholest-4-en-3-one (LVII) (D) on the total lipids concentration in rats brain. The 3β-chloro-5-bromo (LVI) (C) produces significant increase of 110.2% in the cerebrum and 175.1% in the cerebellum, while most significant increase of 209.7% in the total lipid contents is observed in brain stem.

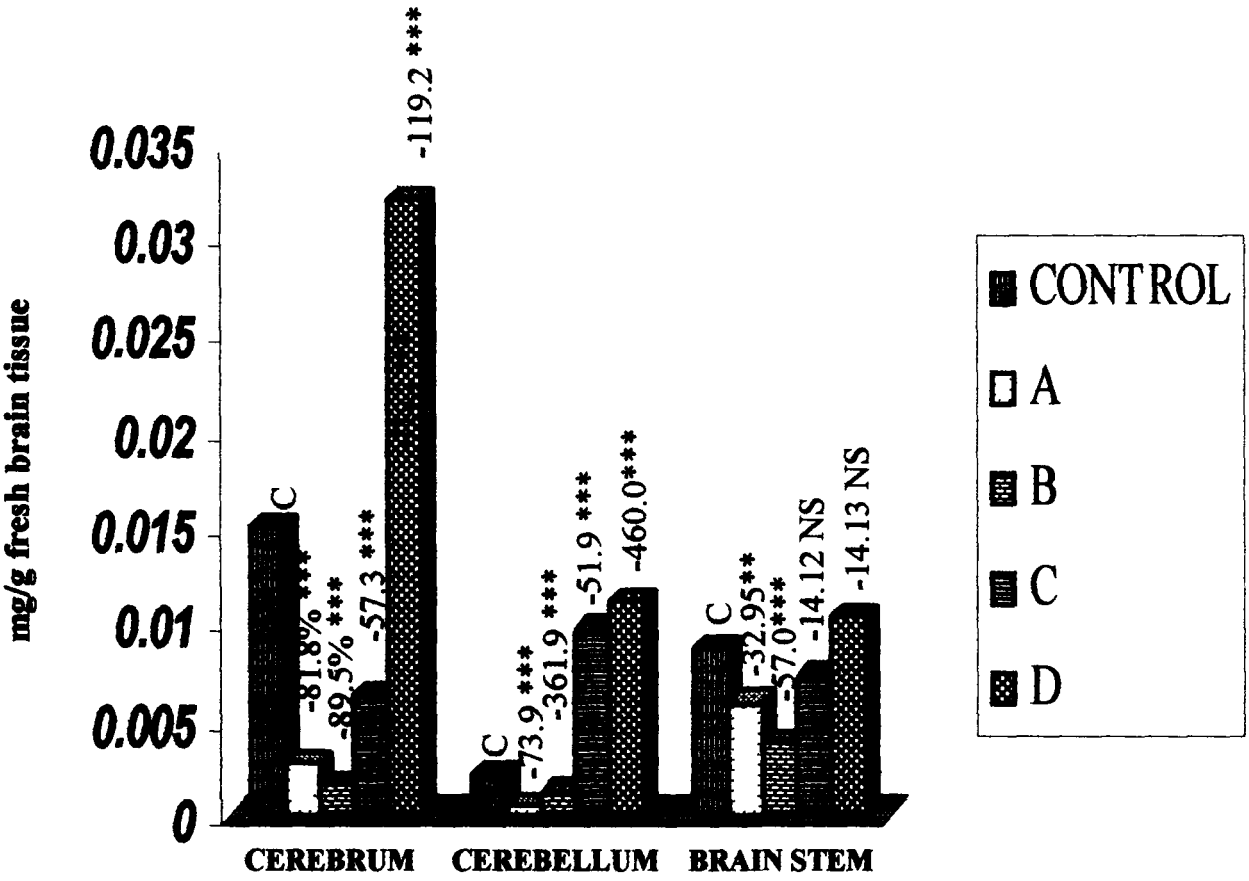
The 6β-Aminopyrimidinocholest-4-en-3-one (LVII) (D) produces significant increase 165.9% and most significant increase of 359.3% in the concentration of total lipids in brain stem and cerebrum respectively while significant decrease of occurs in brain stem –66.23%.

The 3β-acetoxy-5-bromo-6β-hydroxy-5α (LIV) (A) produced significant decrease of -36.7% and significant increase 89.7% in the concentration of total lipids in cerebellum and brain stem and it shows insignificant decrease of –14.33% in cerebrum respectively. The compound (LV) (B) shows significant increase 174.1% and significant decrease of –41.13% in the concentration of total lipids in brain stem and cerebrum respectively while insignificant decrease of –5.67% occur in brain stem.



(Fig. 10)
Histogram of Total Lipids

In the content of cholesterol it shows that 6β-aminopyrimidino (LVII) (D) produces most significant increase 460.0% in cerebellum, while significant decrease is observed –119.2% of cerebrum and 14.13% in brain stem. 3β-Chloro-5-bromo-6β (LVI) (C) produces significant decrease – 361.9% in the concentration of cholesterol in cerebellum and –57.3% in cerebrum, while it produces an insignificant deprivation in brain stem - 14.12%. 3β-Acetoxy-5-bromo (LV) (B) produces significant decrease - 89.7% in cerebrum, -57.8% in brain stem and –51.9% in the concentration of cholesterol in cerebellum. 3β-Acetoxy-5-bromo-6β-hydroxy (LIV) (A) produces significant decrease in the cholesterol level in cerebrum -81.8% in cerebellum -73.9% and in brain stem -32.9%.

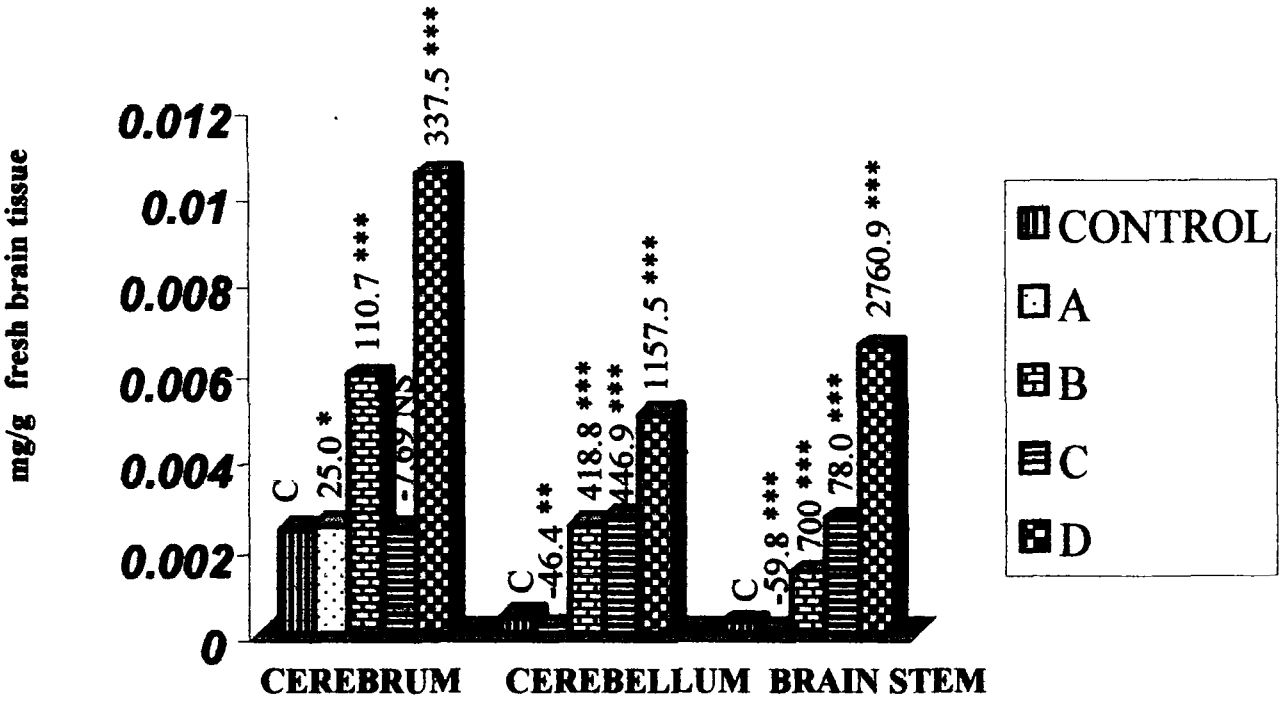


(Fig. 11)

Histogram of Cholesterol

The steroidal derivative 6β-aminopyrimidino (LVII) (D) produces most significant increase in the level of gangliosides in brain stem (2760.9%) and cerebellum (1157.5%) and of cerebrum (337.5%). 3β-chloro-5-bromo (LVI) (C) produces most significant increase in level of gangliosides in brain stem (780.0%), cerebellum (446.9%) and insignificant decrease in cerebrun (-7.69%) is observed. 3β-acetoxy-6-dimethylamino (LV) (B) produces most significant increase in brain stem (700.0%), cerebellum (418.8%) and in cerebrum (110.7%). 3β-acetoxy-5-bromo-6β (LIV) (A) produces significant

decrease in level of gangliosides in brain stem (-59.8%) and in cerebellum (-46.5%). While significant increase is observed (25.0%) in cerebrum.



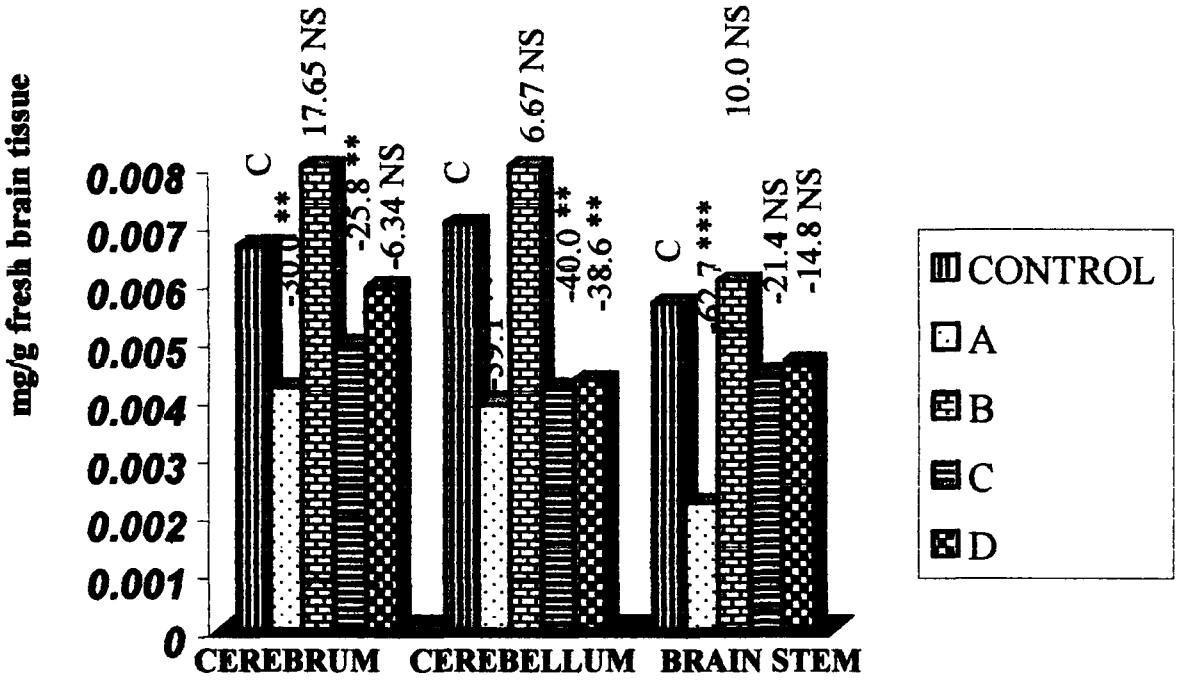
(Fig. 12)

Histogram of gangliosides

The data obtained for the rate of lipid Peroxidation following the administration of four steroidal derivatives. 3β-Acetoxy-5-bromo-6β-hydroxy (LIV) (A) produces significant decrease of 62.7% in brain stem, -39.1% in cerebellum and of cerebrum (-30.0%) is observed.

3β-acetoxy-6-dimethylamino (LV) (B) produces insignificant increase of 17.65% in cerebrum, 10.0% in brain stem and 6.67% is reported in cerebellum of rats brain. 6β-Aminopyrimidino (LVII) (D) produces significant

decrease of –38.6% in cerebellum while insignificant depletion of 14.8% and 6.34% is found in brain stem and cerebrum respectively. 3β-Chloro-5-bromo (LVI) (C) produces significant decrease of 40.0% in cerebellum and of 25.8% in cerebrum. While insignificant decrease of 21.4% is reported in brain stem of rats brain in the study.



(Fig. 13)

Histogram of lipid peroxidation

These steroidal compounds were prepared according to literature procedure characterized by spectral and chemical evidences and comparison with authentic sample in known cases.

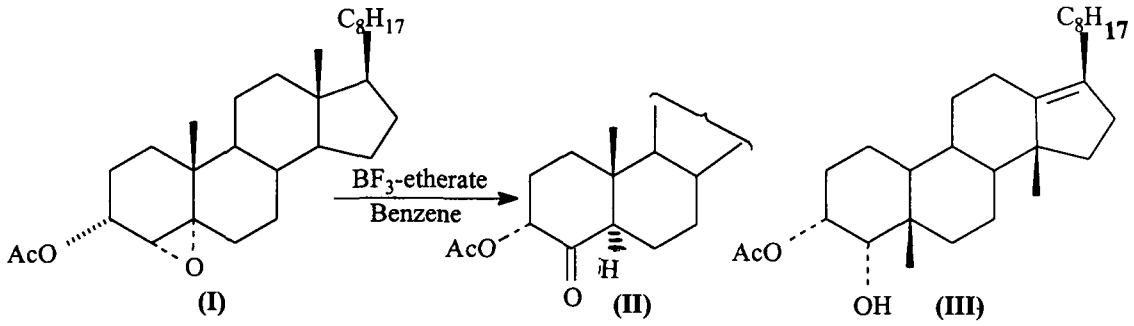
CHAPTER - 1

Synthesis of Steroidal Oxazolines and aziridine (Reaction of Steroidal epoxides)

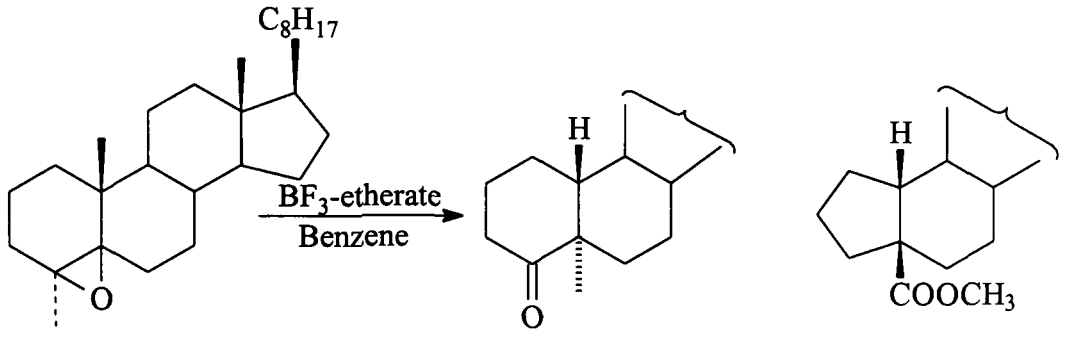
THEORITICAL

Epoxide ring opening reactions have been reported at large in the recent past[1-4]. The epoxide ring is very sensitive and opens generally under mild conditions when it comes in contact with acids or bases[5-8]. A number of papers dealing with the reactions of epoxides with a variety of reagents have appeared where anionic and cationic cleavages of epoxide ring followed by some novel rearrangements in certain cases have been reported.

Blunt et.al.[5] have studied the reaction of 3α-acetoxy-4α, 5-epoxy-5α-cholestane (I) with BF_3-etherate in benzene and obtained 3α-acetoxy-5α-cholestan-4-one (II) along with rearranged product 3α-acetoxy-4α-hydroxy-$\Delta^{13(17)}$ compound (III).

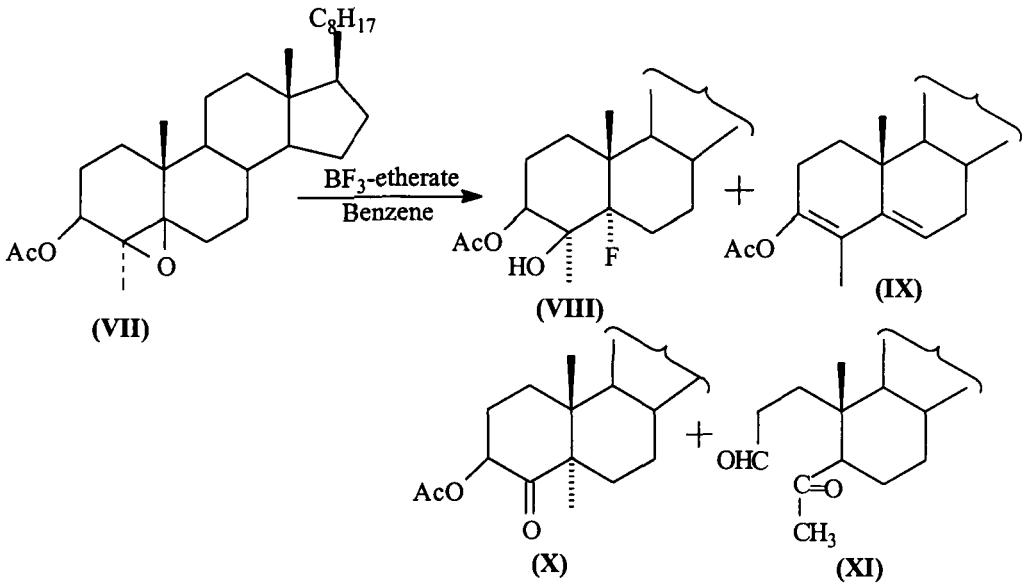


Hortshorn et.al.[6] carriedout the reaction of 4β, 5-epoxy-4α-methyl-5-cholestane (IV) with BF_3-etherate in benzene and obtained 5-methyl-5α-cholestan-4-one (V) and 5β-acetyl-A-nor-cholestane (VI) as the products.



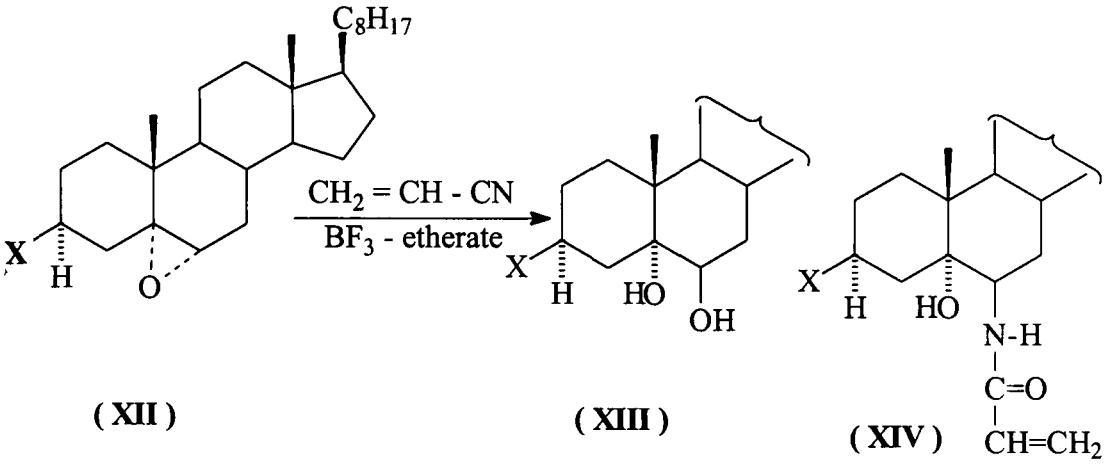
(IV) **(V)** **(VI)**

3β-Acetoxy-5α-fluoro-4α-methylcholestan-4β-ol (VIII), 3-acetoxy-4-methylcholest-3, 5-diene (IX), 3β-acetoxy-5α-methylcholestan-4-one (X) and compound (XI) were obtained when 3β-acetoxy-4β, 5-epoxy-4α-methyl-5β-cholestane (VII) was treated with BF_3-etherate in benzene[7].



(VII) (VIII) (IX) (X) (XI)

The reaction of 5, 6α-epoxy-5α-cholestane (XII-a), its 3β-chloro (XII-b) and 3β-acetoxy analogues (XII-c) with acrylonitrile (BF_3-etherate as catalyst) provided 5, 6β-dihydroxy-5α-cholestane (XII-a), its 3β-chloro (XII- b) and 3β-acetoxy analogue (XII-c) and 5-hydroxy-6β-acrylamido-5α-cholestane (XIV-a), its 3β-chloro (XIV-b) and 3β-acetoxy analogues (XIV-c)[8].



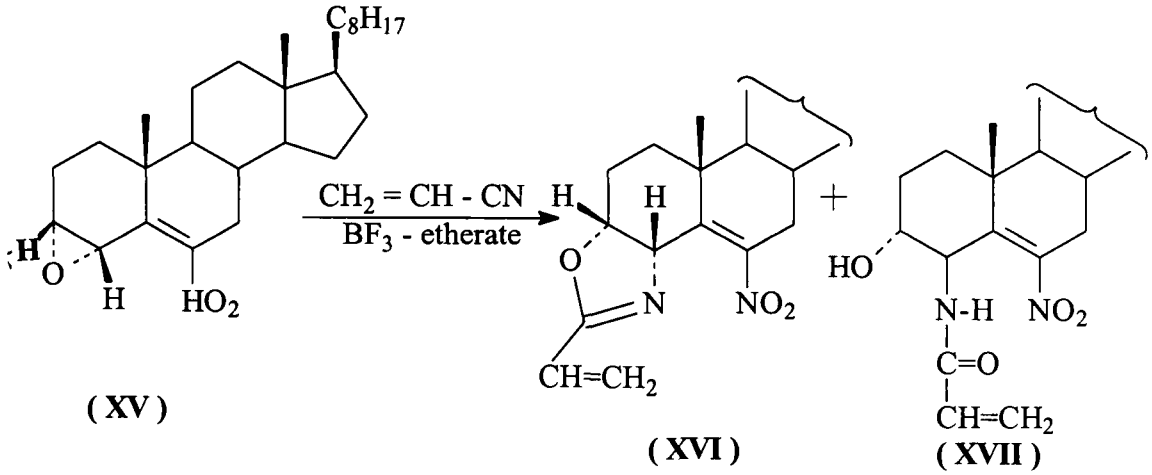
(XII) **(XIII)** **(XIV)**

	X
a	H
b	Cl
c	OAc

	X
(a)	H
(b)	Cl
(c)	OAc

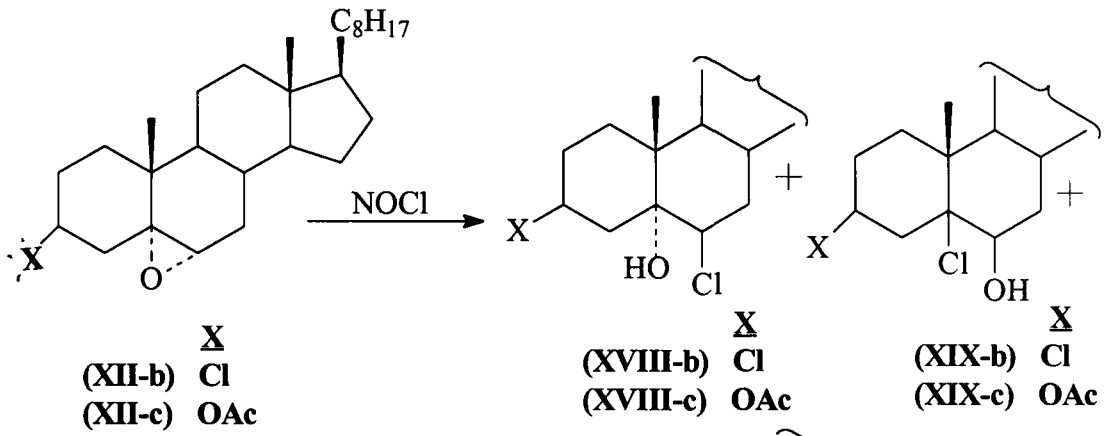
	X
(a)	H
(b)	Cl
(c)	OAc

6-Nitrocholest-5-ene (4α,3α-d)-2′-vinyl-2-oxazoline (XVI) and 3α-hydroxy-4β-acrylamido-6-nitrocholest-5-ene (XVII) were obtained when 3α, 4α-epoxy-6-nitrocholest-5-ene (XV) was treated with acrylonitrile in the presence of BF_3-etherate[9].



(XV) **(XVI)** **(XVII)**

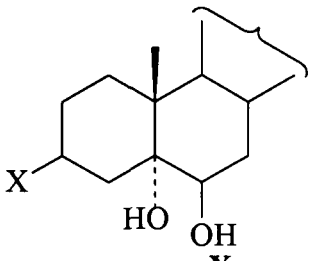
Reaction of 3β-chloro 5,6α-epoxy-5α-cholestane (XII-b) and its 3β-acetoxy analogue (XII-c) with nitrosylchloride gas provided the respective isomeric chlorohydrins (XVIII-b, c) and (XIX-b, c) and diols (XIII-b, c). However, 3α, 4-epoxy-6-nitrocholest-5-ene (XV) on similar treatment with NOCl afforded 3α-chloro-4β-nitrato-6-nitrocholest-5 ene (XX)[10].



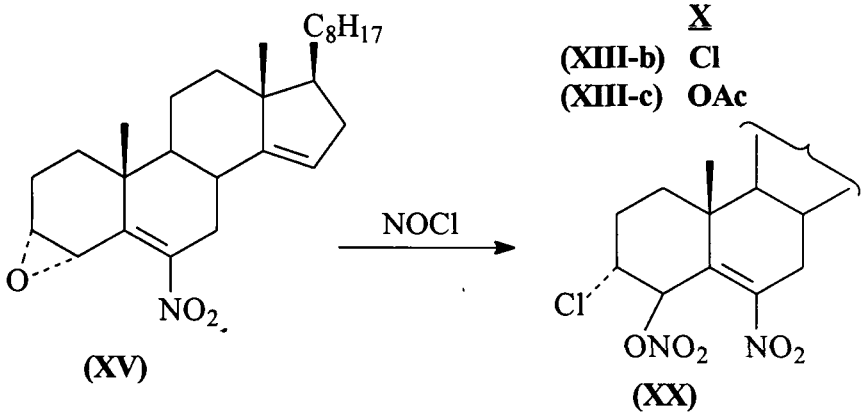
X	
(XII-b)	Cl
(XII-c)	OAc

X	
(XVIII-b)	Cl
(XVIII-c)	OAc

X	
(XIX-b)	Cl
(XIX-c)	OAc



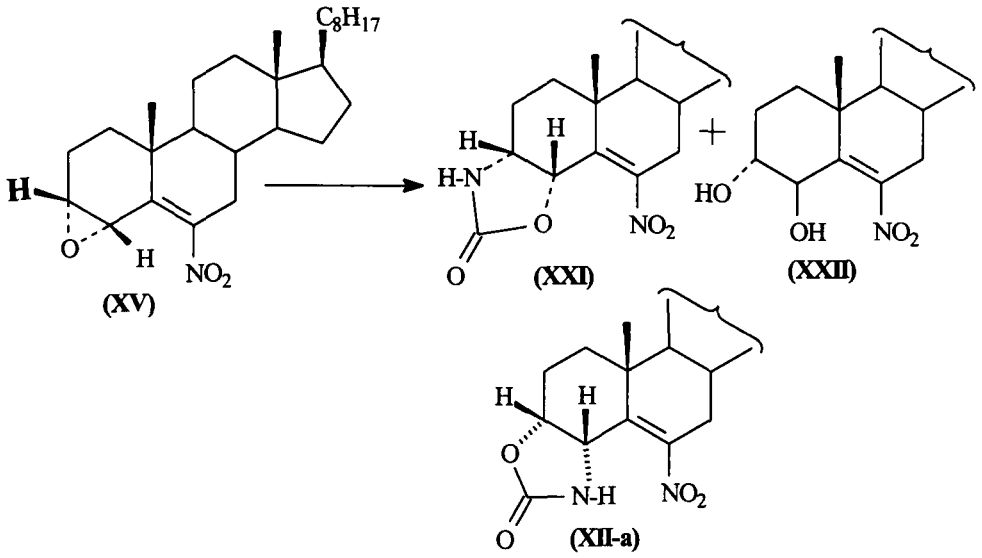
X	
(XIII-b)	Cl
(XIII-c)	OAc



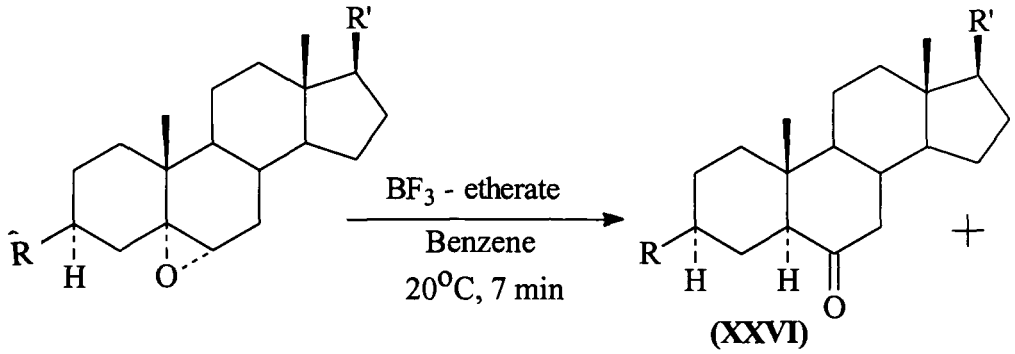
(XV)

(XX)

Oxazolidinone (XXI) and trans-dihydroxy compound (XXII) were obtained when 3α, 4α-epoxy-6-nitrocholest-5-ene (XV) was treated with phenylisocyanate ($AlCl_3$ used as catalyst)[11]. When some epoxide (XV) was treated with urea in DMF, Oxazolidinone (XXI) and its isomer (XXI-a) is obtained[11-a].



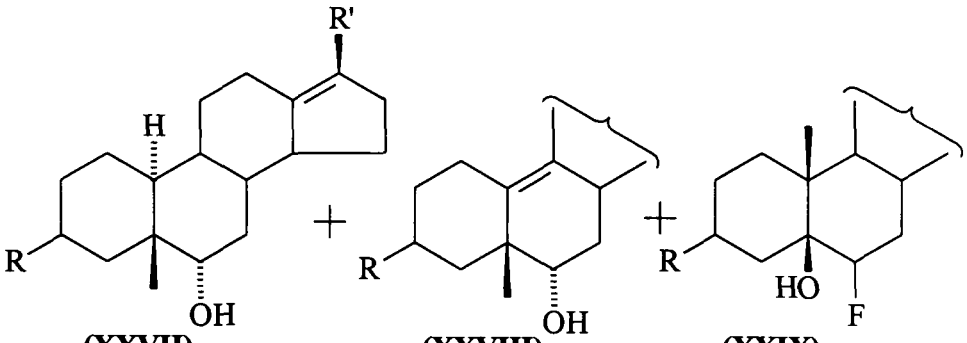
The reaction of 3β-chloro-5,6α-epoxy-5α-cholestane (XII-b), 3β-chloro-5,6α-epoxy-5α-stigmastane (XXIII),its 3β-hydroxy (XXIV) and 3β-acetoxy (XXV) analogues with BF_3-etherate in benzene furnished ketones (XXVI a-d) backbone rearranged products (XXVII a-d), westphalen rearranged products (XXVIII a-d) and fluorohydrins (XXIX a-d)respectively[12a,b].



(XXVI)

	R	R'
(XXII b)	Cl	C_8H_{17}
(XXIII)	Cl	$C_{10}H_{21}$
(XXIV)	OH	$C_{10}H_{21}$
(XXV)	OAc	$C_{10}H_{21}$

	R	R'
(a)	Cl	C_8H_{17}
(b)	Cl	$C_{10}H_{21}$
(c)	OH	$C_{10}H_{21}$
(d)	OAc	$C_{10}H_{21}$



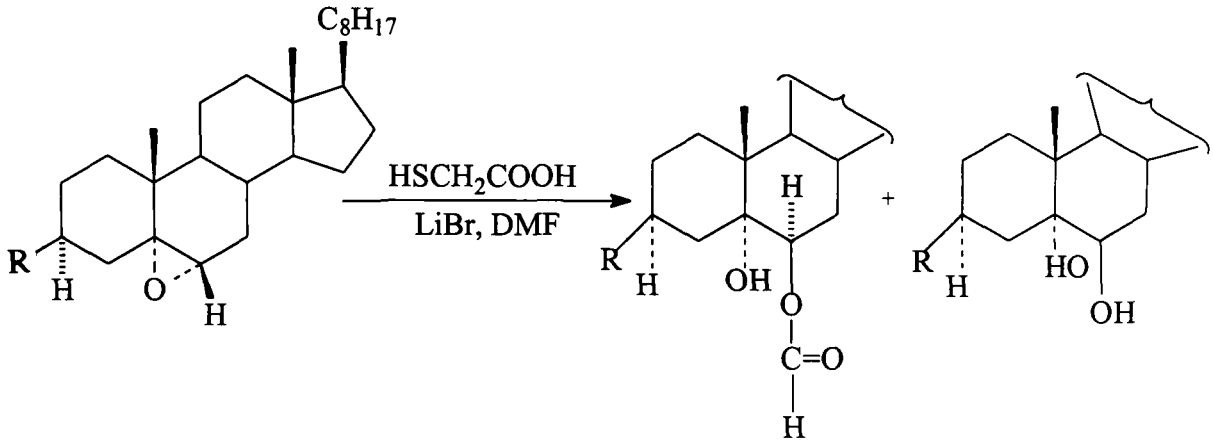
(XXVII) (XXVIII) (XXIX)

R	R'
(a) Cl	C_8H_{17}
(b) Cl	$C_{10}H_{21}$
(c) OH	$C_{10}H_{21}$
(d) OAc	$C_{10}H_{21}$

R	R'
(a) Cl	C_8H_{17}
(b) Cl	$C_{10}H_{21}$
(c) OH	$C_{10}H_{21}$
(d) OAc	$C_{10}H_{21}$

R	R'
(a) Cl	C_8H_{17}
(b) Cl	$C_{10}H_{21}$
(c) OH	$C_{10}H_{21}$
(d) OAc	$C_{10}H_{21}$

The reaction of steroidal epoxides (XXII a-c and XXX) with thioglycolic acid provided steroidal hydroxyformates (XXXI a-d) and diols (XXII a-c and XXXII). The structures of these compounds were established on the basis of spectral evidences, X-ray analysis and comparison with authentic samples in known cases[13].

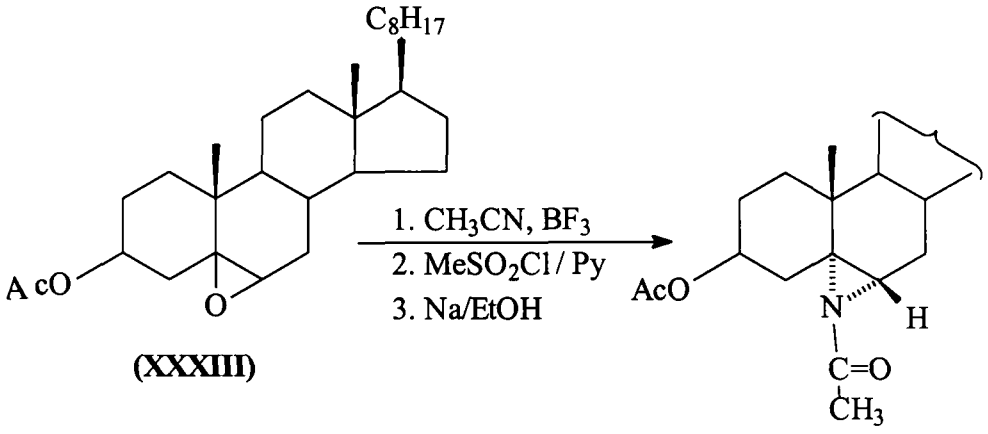


	R
(XXII - a)	H
(XXII - b)	Cl
(XXII - c)	OAc
(XXX)	OH

	R
(XXXI - a)	H
(XXXI - b)	Cl
(XXXI - c)	OAc
(XXXI - d)	OH

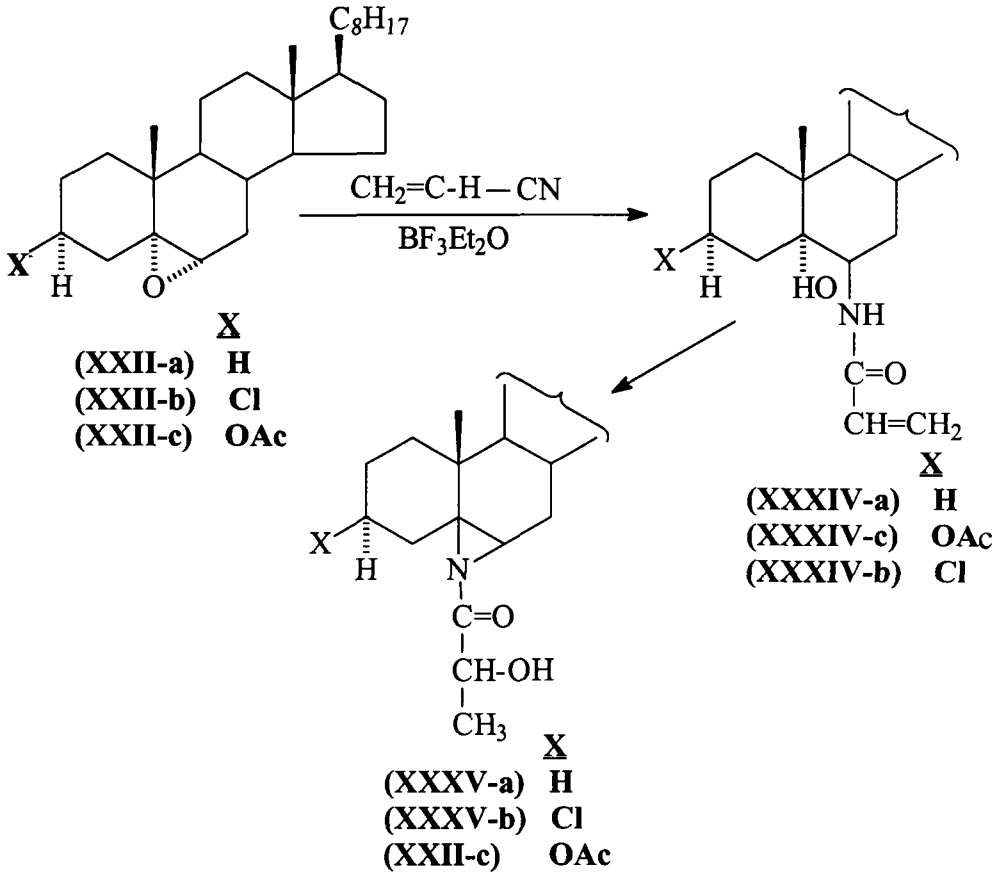
	R
(XIII - a)	H
(XIII - b)	Cl
(XIII - c)	OAc
(XXXII)	OH

Ducker and Lazer reported the formation of aziridine (XXXIII-a) from the β-epoxide (XXXIII) via Ritter reaction[14].



(XXXIII) **(XXXIII-a)**

Recently the synthesis of N-(2`-hydroxy-2`-methyl) acetyl-3β-substituted-5β-cholestanoaziridines (XXXVa-c)from corresponding epoxides (XIIa-c) through the following sequence of reaction was reported[8].

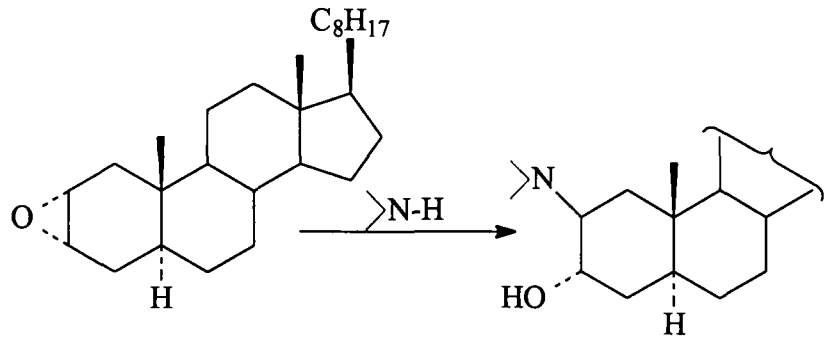


X	
(XXII-a)	**H**
(XXII-b)	**Cl**
(XXII-c)	**OAc**

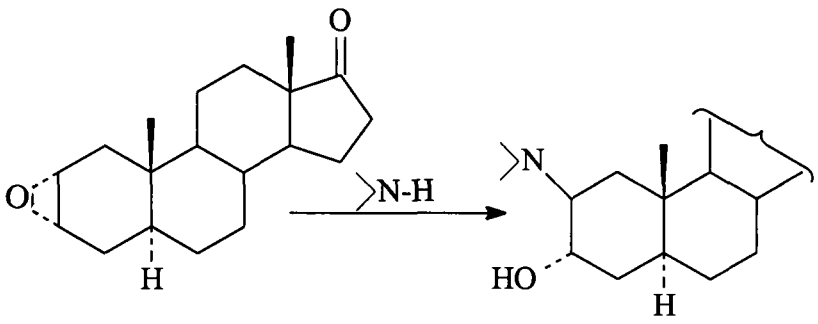
X	
(XXXIV-a)	**H**
(XXXIV-c)	**OAc**
(XXXIV-b)	**Cl**

X	
(XXXV-a)	**H**
(XXXV-b)	**Cl**
(XXII-c)	**OAc**

2α, 3α-Epoxy-5α-cholestane (XXXVI) when treated with dimethyl-amine afforded 2β-dimethylamino-5α-cholestan-3α-o1 (XXXVII). A similar synthesis of 2β-dimethylamino-3α-hydroxy-5α-androstan-17-one (XXXIX) from 2α,3α-epoxy-5α-androstan-17-one (XXXVIII) has been reported[15].



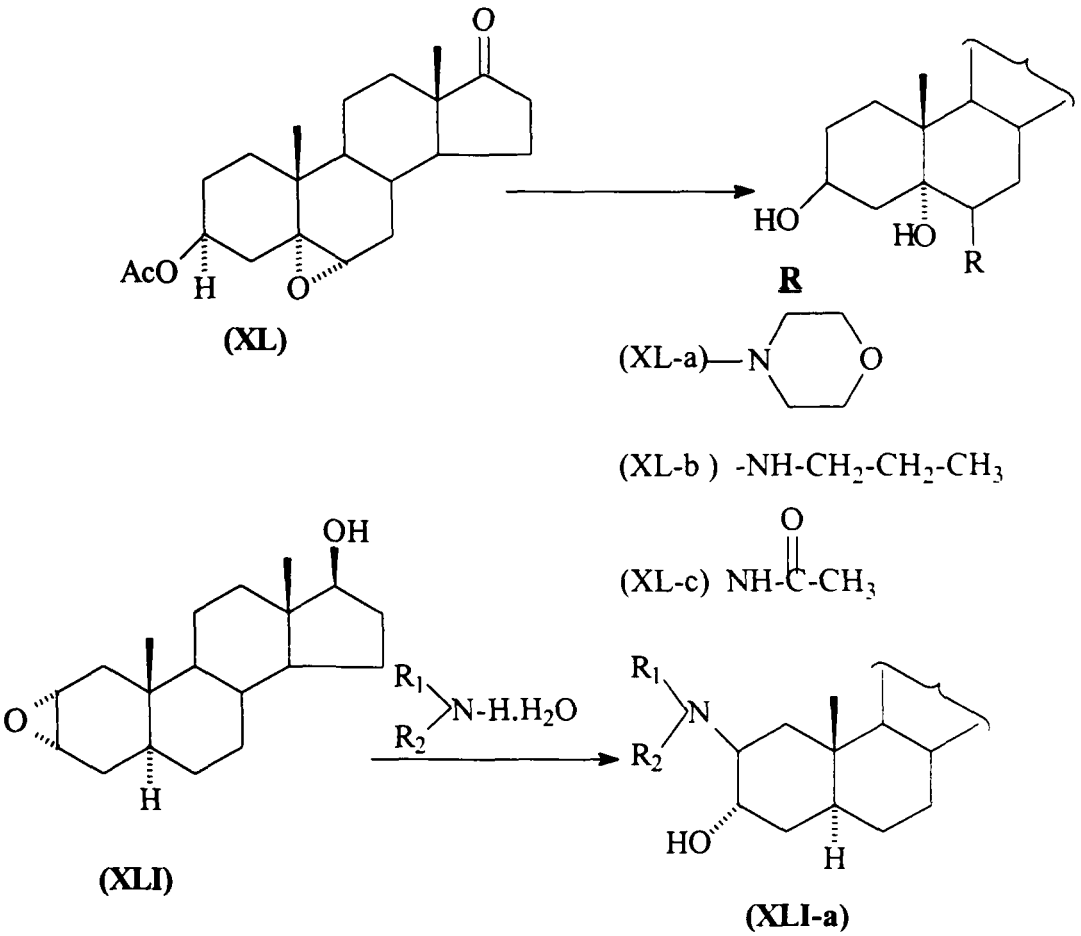
(XXXVI) **(XXXVII)**



(XXXVIII) **(XXXIX)**

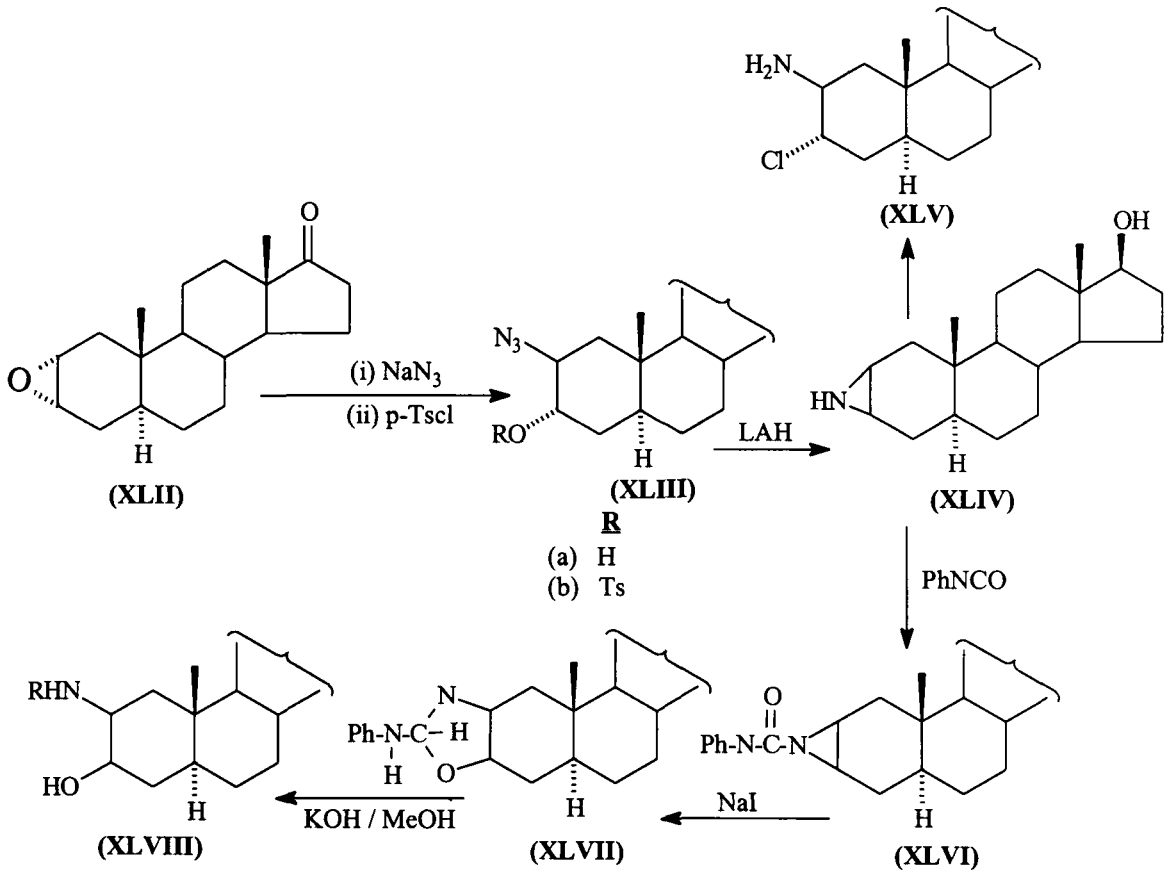
Hewett and coworkers[16] reported the preparation of aminosteroids (XLa-c) from 3β-acetoxy-5, 6α-epoxy-5α-androstan-17-one (XL). Condensation of 2α, 3α-epoxy-5α-cholestan-17β-ol(XLI) with secondary

amine in water gave the corresponding 2β-amino-5α-androstane-3α, 17β-deiol[16] (XLI – a).



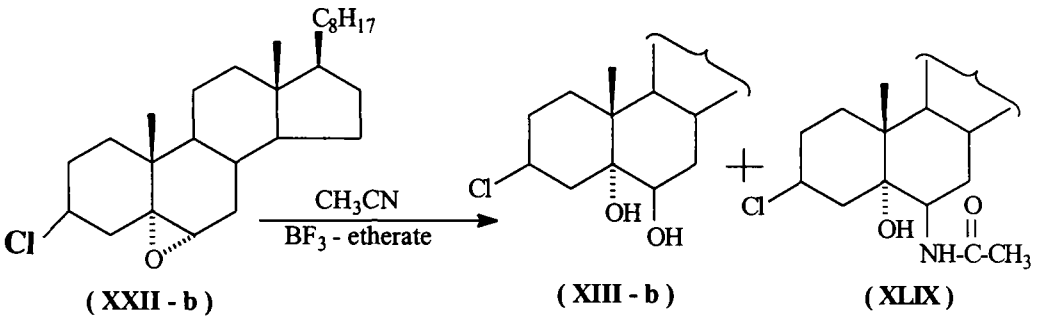
Ponsold and Preibsch[17] synthesized 2β, 3β-imino-5α-androstan-17β-ol (XLIV) from 2α, 3α-epoxy-5α-cholestan-17-one (XLII) via the corresponding azidoalcohol tosylate (XLIII) and converted into 2β-amino-3α-chloro-5α-

androstan-17-ol (XLV). 2β-Amino-3β-hydroxy derivative (XLVIII) was prepared from aziridine (XLVI).

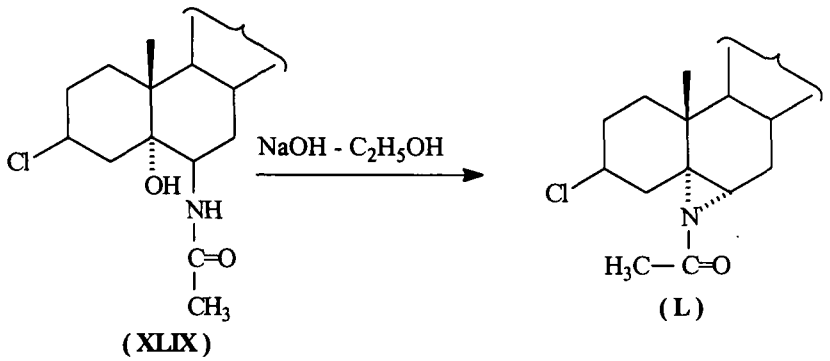


Ritter reaction of 3β-chloro-5, 6α-epoxy-5α-cholestane (XXII-b) in acetonitrile-BF_3 etherate provided 3β-chloro-5, 6β-dihydroxy-5α-cholestane (XIII-b) and 3β-chloro-5-hydroxy-6β-acetylamino-5α-cholestane (XLIX).

The (XLIX) on treating with $NaOH\text{-}C_2H_5OH$ provided 1′-acetyl-3β-chloro-5β-cholestano [5, 6-d]-azirine[18] (L).

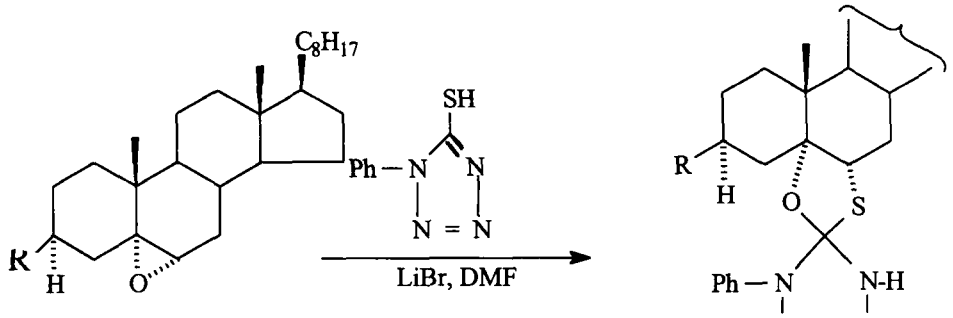


(XXII - b) (XIII - b) (XLIX)



(XLIX) (L)

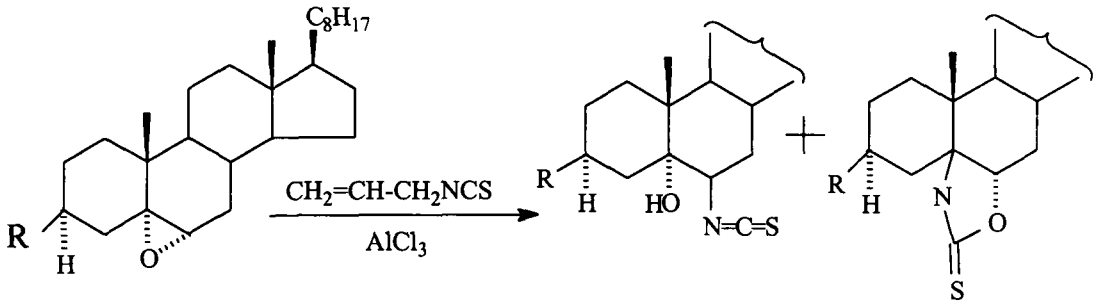
The reactions of epoxides (XIIa-c) with 1-phenyl-1H-tetrazole-5-thiol in the presence of lithium bromide and dimethylformamide provided (LIVa-c)[19].



R
(XII - a) H
(XII - b) Cl
(XII - c) OAc

R
(LI - a) H
(LI - b) Cl
(LI - c) OAc

5, 6α-Epoxy-5α-cholestane (XII-a), its 3β-chloro (XII-b) and 3β-acetoxy (XII-c) analogues when treated with allyl isothio cyanate (using $AlCl_3$ as catalyst) provided 5-hydroxy-5α-cholestan-6β-Isothiocyanates (LIIa-c) and oxazolidin-2′-thiones (LIIIa-c)[20].

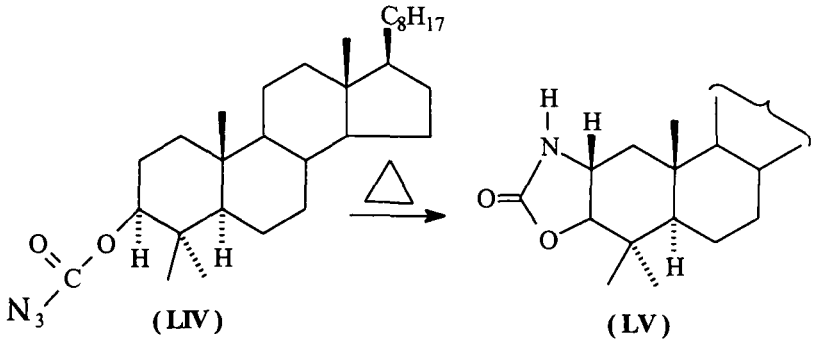


R
(XII - a) H
(XII - b) Cl
(XII - c) OAc

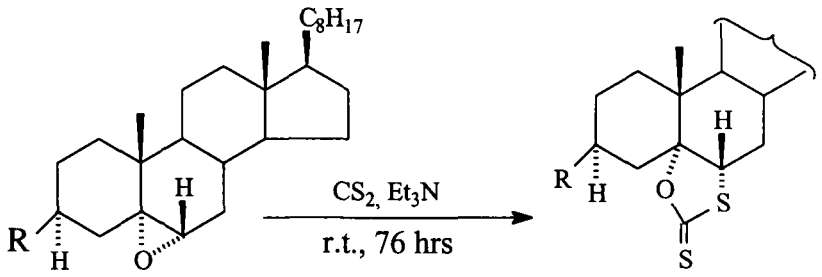
R
(LII - a) H
(LII - b) Cl
(LII - c) OAc

R
(LIII - a) H
(LIII - b) Cl
(LIII - c) OAc

On thermolysis 3β-lanostanyl azidoformate (LIV) gave oxazolidinone (LV)[21].



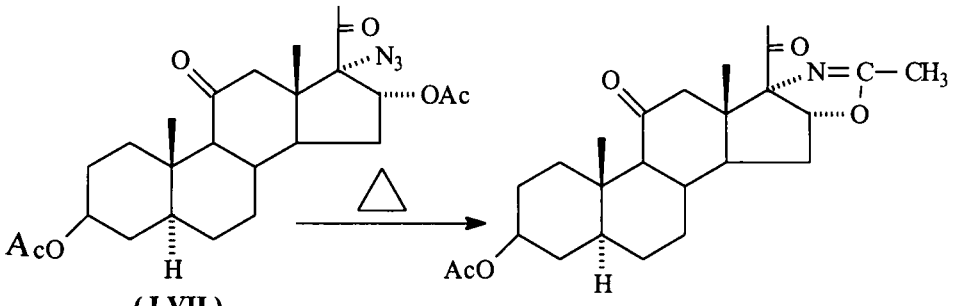
The reaction of 5, 6α-epoxy-5α-cholestane (XII-a), its 3β-chloro (XII-b) and acetoxy (XII-c) analogues with carbon disulphide in triethylamine furnished 5α-cholestano-[6α, 5-d]-1′, 3′-oxathiolane-2-thione (LVI-a) its chloro (LVI-b) and acetoxy analogues (LVI-c)[22].



R	
(XII - a)	H
(XII - b)	Cl
(XII - c)	OAc

R	
(LVI - a)	H
(LVI - b)	Cl
(LVI - c)	OAc

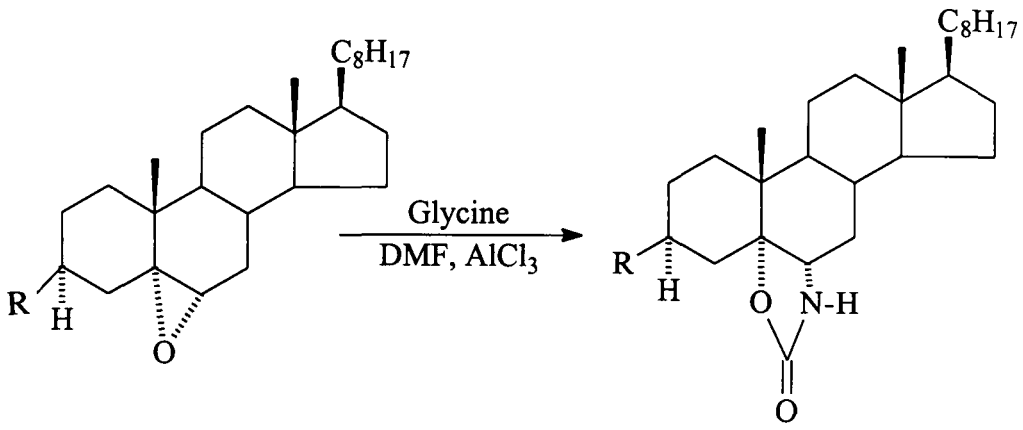
Nathansohn and coworkers[23] converted 17α-azido-5α-pregnan-3β, 16β-diol-11, 20-dione-3, 16-diacetate (LVII) into 5α-pregnan-3β-ol-11, 20-dione-[17α, 16α-d]-2′-methyl oxazoline (LVIII).



(LVII) **(LVIII)**

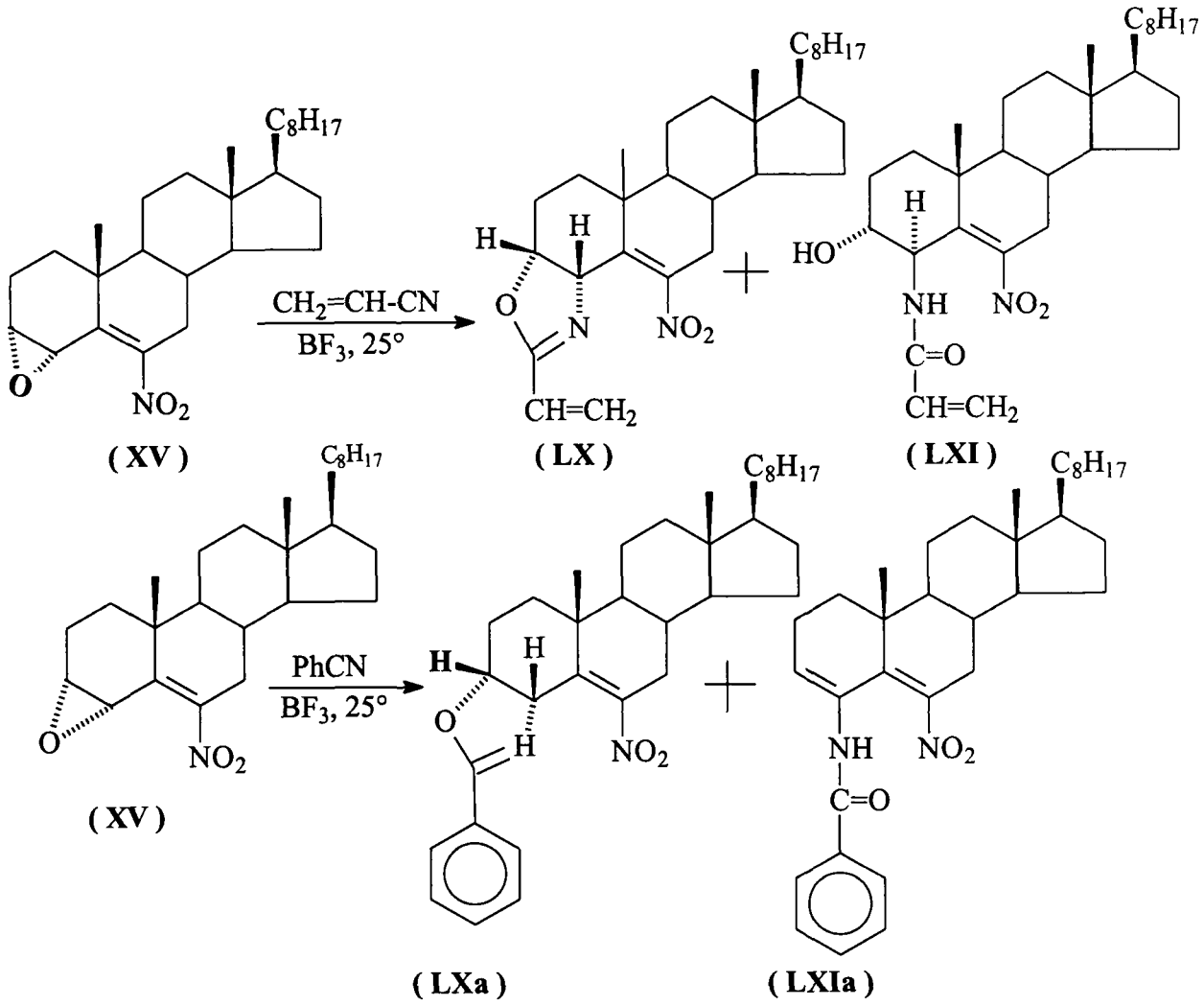
DISCUSSION

Reactions of steroidal epoxides with appropriate regents leading to the synthesis of steroidal oxazolidinones[11,11-a], Oxazoles[24], Oxazolines[9,23], Oxathiolane thiones[20] and aziridines[8,14] have been reported from our laboratories and from other research centres because of their pharmaceutical importance[25-29] which include inflammatory[25], hypertensive[26], tranquilizing[27] and carcinostatic[28,29] activities besides other general ring opening reactions[5-7,12a,b] of epoxides. Recent publication[30] from our laboratories deal with the synthesis of steroidal oxazolidinones (LIXa-c).

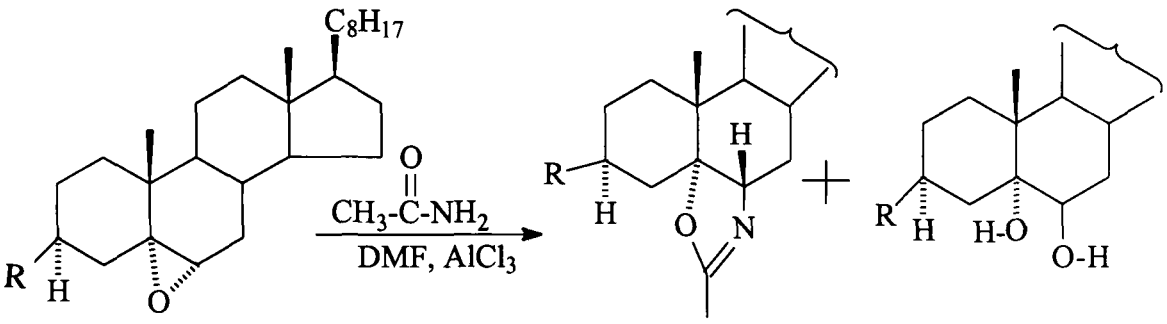


R	R
(XII-a) H	(LIX-a) H
(XII-b) Cl	(LIX-b) Cl
(XII-c) OAc	(LIX-c) OAc

Our previous work relates the synthesis of steroidal oxazolines, such as 6-nitrocholest-5-eno[3α, 4α-d]-2′-vinyl-2-oxazoline (LX)[9] and 6-nitrocholest-5-eno [3α, 4α-d]-2′-phenyl-2-oxazoline (LXa)[31] from 3α, 4α- epoxy-6-nitro cholest-5-ene (XV).



In continuation to the synthesis of steroidal oxazolidines, we have subjected 5, 6α-epoxy-5α-cholestane (XII-a), it 3β-chloro (XII-b) and acetoxy (XII-c) analogues to the reactions of acetamide in DMF ($AlCl_3$ as catalyst) which afforded 5α-cholestano (5, 6α-d)-2′-methyl-2- oxazoline (LXII-a), its 3β-chloro (LXII-b) its acetoxy (LXII-c) analogues and 5, 6β-dihydroxy-5α-cholestane (XII-a) its 3β-chloro (XII-b), 3β-acetoxy analogues (XII-c).



	R
(XII - a)	H
(XII - b)	Cl
(XII - c)	OAc

	R
(LXII - a)	H
(LXII - b)	Cl
(LXII - c)	OAc

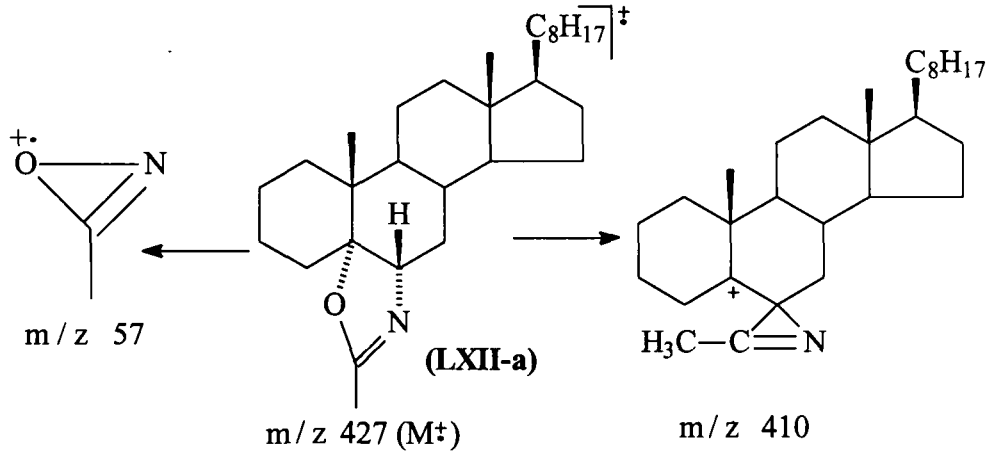
	R
(XIII - a)	H
(XIII - b)	Cl
(XIII - c)	OAc

Reaction of 5, 6α-epoxy-5α-cholestane (XII-a) with acetamide – DMF (anhydrous $AlCl_3$ as catalyst) : 5α-Cholestano (5, 6α-d)-2′-methyl-2-oxazoline (LXII-a) and 5, 6β-dihydroxy-5-cholestane (XIII-a) :

5, 6α-Epoxy-5α-cholestane (XII-a) was dissolved in DMF and required amount of acetamide was added in portions. Small amount of anhydrous $AlCl_3$ was added as catalyst. After the completion of the reaction, the reaction mixture was work up. The solvent was removed and the residue thus obtained was chromatographed over silica gel. Elution with petroleum ether : ether provided two soild compounds m.p. 101° and 124 - 125°.

Characterization of compound, m.p. 101° as 5α-Cholestano (5, 6α-d)-2′-methyl-2-oxazoline (LX) :

The compound, m.p. 101° was correctly analysed for $C_{29}H_{49}NO$. The IR spectrum of the compound exhibited bands at 1680 (C=N), 1350 (C-N) and 1050 cm^{-1} (C-O). ^{1}H-NMR spectrum gave peaks at δ 4.78 (1H, dd, Jaa = 11 Hz, Jae = 4 Hz, H-6β), 2.13 (s, 3H, CH_3-C=N-), 1.01 (C10-CH_3), 0.68 (C13-CH_3), 0.95 and 0.85 (side chain methyl protons). The mass spectrum of the compound gave molecular ion at m/z 427 ($M^{\dot{+}}$) followed by some other important fragment ions such as at m/z 410 and m/z 57 and other fragment ions lower mass peaks.



On the basis of above spectral evidences, the structure of the compound, m.p. 101 is characterized as 5α-cholestano [5, 6α-d]-2′-methyl-2-Oxazoline (LXII-a).

Characterization of compound, m.p. 124-125° as 5, 6β-Dihydroxy 5α-cholestane (XIII-a) :

The compound m.p. 124-125° (reported[38] m.p. 125.5°) was analysed correctly for $C_{27}H_{48}O_2$ and characterized as 5, 6β-dihydroxy-5α-cholestane (XIII-a) on the basis of m.p., m.m.p., TLC, IR and ^{1}H-NMR which were found with the authentic sample.

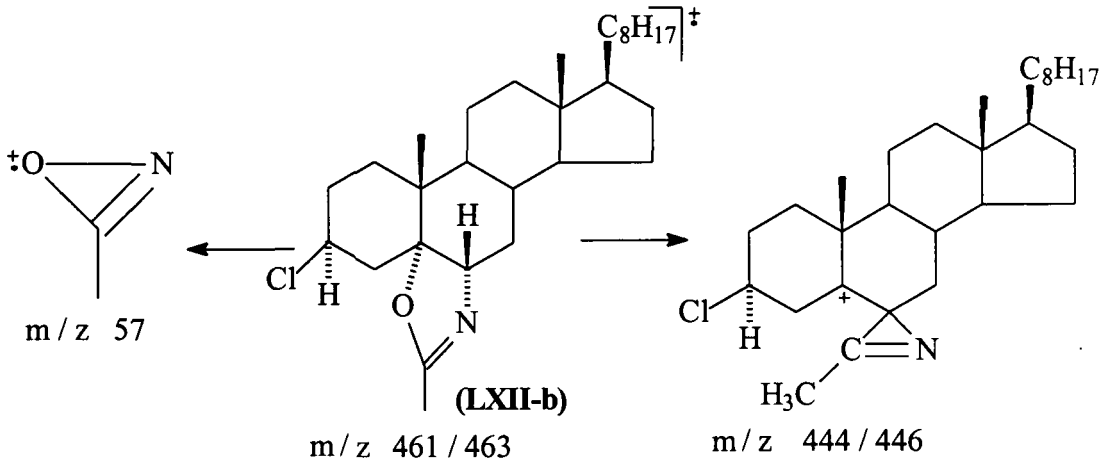
Reaction of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) with acetamide – DMF (anhydrous $AlCl_3$ as catalyst) : 3β-Chloro-5α-cholestano (5, 6α-d)-2′-methyl-2-oxazoline (LXII-b) and 3β-chloro-5, 6β-dihydroxy-5α-cholestane (XIII-b) :

3β-Chloro-5, 6α-epoxy-5α-cholestane (XII-b) was dissolved in DMF and required amount of acetamide was added in portions. Small amount of anhydrous $AlCl_3$ was added as catalyst. After the completion of reaction and usual work up and column chromatography over silica gel two compounds m.p. 110° and 124° were obtained.

Characterization of compound, m.p. 110° as 3β-Chloro-5α-cholestano [5, 6α-d]-2′-methyl-2-oxazoline (LXII-b) :

The compound, m.p. 110° was analysed correctly for $C_{29}H_{48}NOCl$ (positive Beilstein test). IR spectrum of the compound exhibited bands at 1690 (C=N), 1360 (C-N), 1060 (C-O) and 710 cm^{-1} (C-Cl). ^{1}H-NMR spectrum gave peaks at δ 4.80 (1H, dd, Jaa = 12, J = 4.5 Hz; H-6β), 3.90 (mc, 1H, W½ = 16 Hz, H-3α)[33], 2.15 (s, 3H, CH_3-C=N-), 1.02 (C10-CH_3), 0.65 (C13-CH_3), 0.98,

0.88 (side chain methyl protons). The mass spectrum of the compound gave molecular ion peaks m/z at 461/468 ($M^{+\cdot}$) followed by important fragment ions at m/z 444/446, m/z 57 and some lower mass peaks.



On the basis of above spectral evidences, the structure of the compound, m.p. 110° was confirmed as 3β-chloro-5α-cholestano [5, 6α-d]-2′-methyl-2-oxazoline (LXII-b).

Characterization of compound, m.p. 124° as 3β-Chloro-5, 6β-dihydroxy-5α-cholestane (XIII-b) :

The compound, m.p. 123° (reported[37] m.p. 125-26°) was analysed correctly for $C_{27}H_{47}O_2Cl$. The physical and spectral values (m.p., m.m.p.,

TLC, IR and ^{1}H-NMR) of the compound m.p. 124° were found identical with authentic sample of 3β-chloro-5, 6β-dihydroxy-5α-cholestane (XII-b).

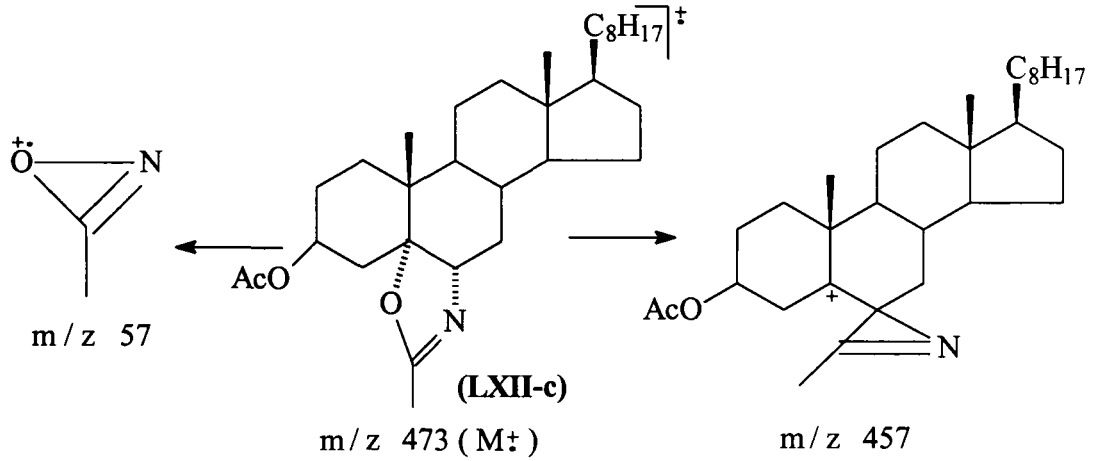
Reaction of 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) in DMF with acetamide (anhydrous $AlCl_3$ used as catalyst) :3β-Acetoxy-5α-cholestano [5,6α-d]-2′-methyl-2-oxazoline (LXII-c) and 3β-acetoxy-5, 6β-dihydroxy-5α-cholestane (XIII-c) :

Acetamide in small portions was added to the solution of 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) dissolved in DMF (anhydrous $AlCl_3$ was added as catalyst). After the completion of the reaction and usual work up and column chromatography over silica gel, two compounds, semi solid and m.p. 208° were obtained.

Characterization of semi solid as 3β-Acetoxy-5α-cholestano [5, 6α-d]-2′-methyl-2-oxazoline (LXII-c) :

The compound obtained as semi solid was analysed correctly for $C_{31}H_{51}NO_3$. The IR spectrum of the compound gave bands at 1735 (CH_3-COO-), 1685 (C=N), 1360 (C-N), 1270, 1030 cm^{-1} (C-O). ^{1}H-NMR spectrum

of the compound gave signals at δ 4.40 (mc, 1H, W½ = 10 Hz, H-6β)[33], 4.10 (mc, 1H, 17 Hz, H-3α)[33], 2.3 (s, 3H, CH_3-C=N-), 2.10 (s, CH_3-COO-), 1.02 (C10-CH_3), 0.69 (C13-CH_3), 0.94 and 0.86 (side chain methyl protons). The mass spectrum of compound gave molecular ion peak at m/z 473 ($M^{+\cdot}$) followed by important fragment ions at m/z 456, m/z 57and fragment ions of



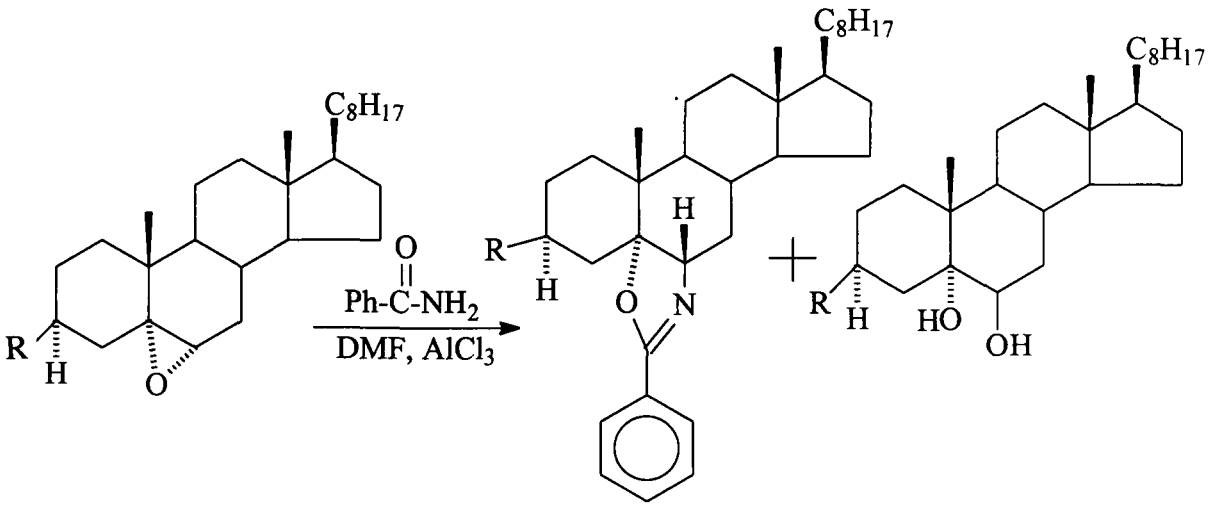
lower mass.

On the basis of above analytical and spectral evidences, the compound obtained as semi solid is identified as 3β-acetoxy-5α-cholestano [5, 6α-d]-2′-methyl-2-Oxazoline (LXII – c).

Characterization of compound m.p. 208° as 3β-Acetoxy-5, 6β-dihydroxy-5α-cholestane (XIII-c) :

The compound, m.p. 208 (reported[32] m.p. 209°) was analysed for $C_{29}H_{50}O_4$ and characterized as 3β-acetoxy-5, 6β-dihydroxy-5α-cholestane (XIII-c) on the basis of m.p., m.m.p., TLC, IR and ^{1}H-NMR mass spectral data which were found identical with authentic sample[32].

Reaction of benzamide with 5, 6α-epoxy-5α-cholestane (XII-a), its 3β-chloro (XII-b) and 3β-acetoxy (XII-c) analogues in DMF (anhydrous $AlCl_3$ as catalyst) afforded 5α-cholestano-(5, 6α-d)-2′-phenyl-2-oxazolidine (LXIV-a), its 3β-chloro (LXIV-b) and acetoxy (LXIV-c) analogues and diols (XIII a-c).



	R
(XII - a)	H
(XII - b)	Cl
(XII - c)	OAc

	R
(LXIII-a)	H
(LXIII-b)	Cl
(LXIII-c)	OAc

	R
(XIII - a)	H
(XIII - b)	Cl
(XIII - c)	OAc

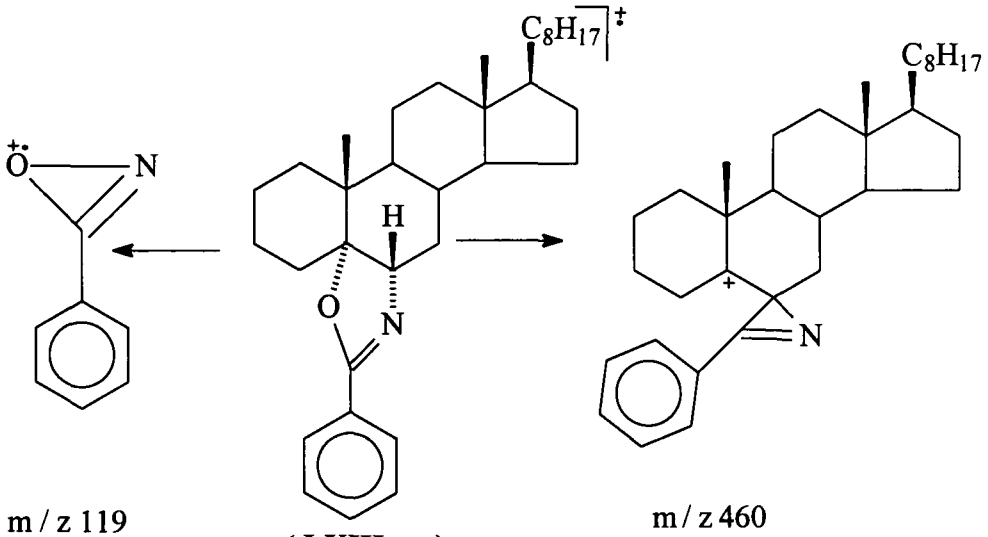
Reaction of 5, 6α-epoxy-5α-cholestane (XII-a) in DMF with benzamide (anhydrous $AlCl_3$ used as catalyst) : 5α-Cholestano [5, 6α-d]-2′-phenyl-2-oxazoline (LXIII-a) and 5, 6α-dihydroxy-5α-cholestane (XIII-a) :

In 5, 6α-epoxy-5α-cholestane (XII-a) dissolved in DMF was added in portions benzamide (anhydrous $AlCl_3$ was used as catalyst). After the completion of the reaction, the solvent was removed and the residue thus obtained was chromatographed over silica gel. Two compounds m.p. 134° and 108° were obtained.

Characterization of the compound, m.p. 134 as 5α-Cholestano-[5, 6α-d]-2′-phenyl-2-oxazoline (LXIII-a).

The compound, m.p. 134° was analysed correctly for $C_{34}H_{51}NO$. The IR spectrum of the compound gave bands at 3100 – 300^{O}(C-H, stretch, aromatic), 1680 (C=N), 1600 – 1590 (C=C, aromatic), 1360 (C-N) and 1050 cm^{-1} (C-O). ^{1}H-NMR spectrum of the compound gave peaks at δ 7.7 (br mc, 5 aromatic protons), 3.5 dd (1H, J = 10 Hz; J = 3.5 Hz; H-6β)[33], 1.2 (C10-CH_3),

0.73 (C13-CH_3), 0.98, 0.85 (side chain methyl protons). Further support for the structure of the compound was given by mass spectrum which contains m/z 477 ($M^{+\cdot}$) followed by important fragment ions at m/z 460 and m/z 119 and fragment ions of lower mass.



m / z 119

(LXIII - a)
m / z 477 ($M^{+\cdot}$)

m / z 460

On the basis of analytical spectral evidence (m.p., m.m.p. TLC, IR, ^{1}H-NMR and mass spectral data) the compound, m.p. 134° is characterized as 5α-cholestano [5, 6β-d]-2′-phenyl-2-oxazoline (LXIII – a).

Characterization of compound, m.p. 108° as 5, 6β-Dihydroxy-5α-cholestane (XIII-a) :

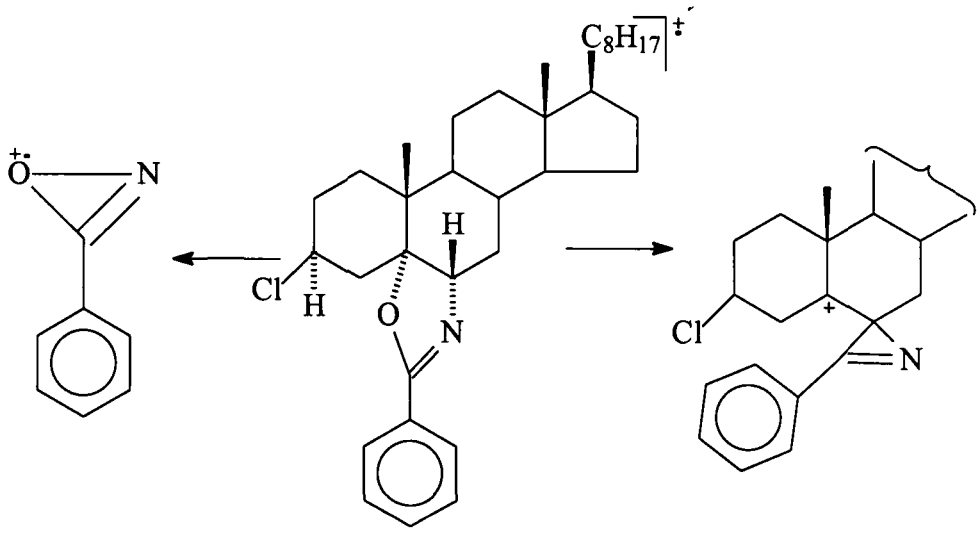
The compound, m.p. 108° was confirmed as 5, 6β-dihydroxy-5α-cholestane (XIII-a) by comparison with authentic sample of the diol[32].

Reaction of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) in DMF with benzamide (anhydrous $AlCl_3$ as catalyst) : 3β-Chloro-5α-cholestano [5, 6α-d]-2′-phenyl-2-oxazoline (LXIII-b) and 3β-chloro-5, 6β-dihydroxy-5α-cholestane-5α-cholestane (XIII-b) :

Benzamide in small portion is added in 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) dissolved in DMF, small amount of anhydrous $AlCl_3$ is also added as catalytic agent. After the completion of reaction, the solvent was removed under reduced pressure and the residue thus obtained was chromatographed over silica gel affording two compounds m.p. 157° and 125°.

Characterization of compound, m.p. 157° as 3β-Chloro-5α-cholestano [5, 6α-d]-2′-phenyl-2-oxazoline (LXIII-b) :

The compound, m.p. 157° was analysed correctly as $C_{34}H_{50}NOCl$ (positive Beilstein Test). The IR spectrum of the compound showed bands at 3150 – 3070 (C-H, stretch, aromatic), 1685 (C=N), 1620(C=C), 1365 (C-N), 1060 (C-O) and 715 cm^{-1} (C-Cl). ^{1}H-NMR spectrum of the compound gave peaks at δ 7.78 (brmc, 5H, aromatic protons), 3.90 (1H, dd, Jaa = 11 Hz, Jae 3.8 Hz, H-6β)[33], 3.95 (mc, 1H, W½ = 18 Hz, H-3α)[34]., 1.10 (C10-CH_3), 0.70 (C13-CH_3), 0.92, 0.87 (side chain methyl protons). The mass spectrum of the compound gave molecular ions at m/z 523/525 ($M^{+\cdot}$) followed by two diagnostic fragment ions peaks at m/z 506/508, m/z 119 and fragment ions of lower mass.



m / z 119

(LXIII - b)
m / z 523 / 525 (M+)

m / z 506 / 508

Characterization of compound, m.p. 125° as 3β-Chloro-5, 6β-dihydroxy-5α-cholestane (XIII-b).

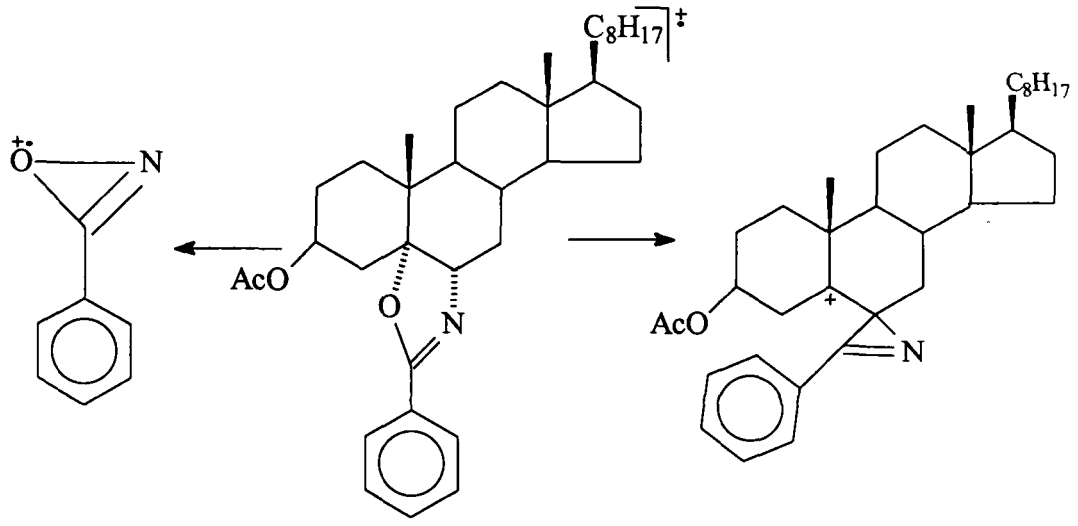
The structure of the compound, m.p.123° was established as 3β-chloro-5,6β-dihydroxy-5α-cholestane (XIII-b) by comparison of m.p., m.m.p., TLC, IR and ^{1}H-HMR with authentic sample[37].

Reaction of 3β-acetoxy-5,6α-epoxy-5α-cholestane (XII-c) in DMF with benzamide (anhydrous $AlCl_3$ as catalyst) : 3β-Acetoxy-5α-cholestano [5,6 α-d]-2′-phenyl-2-oxazoline(LXIII-c) and 3β-acetoxy-5,6β-dihydroxy-5α-cholestane. (XIII-c) :

The 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) was dissolved in DMF. Required amount of benzamide is added to it in portions in the presence of anhydrous $AlCl_3$ (catalyst). Removal of the solvent after the completion of reaction, gave an oil which was chromatographed over silica gel. Two compounds m.p. 220° and 209° were obtained.

Characterization of compound, m.p. 220° as 3β-acetoxy-5α-cholestano [5, 6α-d]-2′-phenyl-2oxazoline (LXIII-c) :

The compound, m.p. 220° was analysed correctly for $C_{36}H_{53}NO_3$. The IR spectrum of the compound gave bands at 3150 – 3060 (C-H, stretch, aromatic), 1740 (CH_3COO), 1665 (C=N), 1615 – 1585 (C=C), 1365 (C-N), 1260 – 1025 cm^{-1} (C-O). ^{1}H-NMR spectrum of the compound gave peaks at δ 7.9 (brmc, 5 aromatic protons), 4.35 (mc, 1H, W½ = 12 Hz, H-6β)[33], 4.25 (mc, 1H, 18 Hz, H-3α)[33], 2.15 (s, CH_3COO), 1.12 (C10-CH_3), 0.72 (C13-CH_3), 0.96 and 0.85 (side chain methyl protons). The mass spectrum of the compound gave molecular ion peak at m/z 547 (M^+) followed by some important peaks at m/z 530, m/z 119 and fragment ions of lower mass.



m/z 119

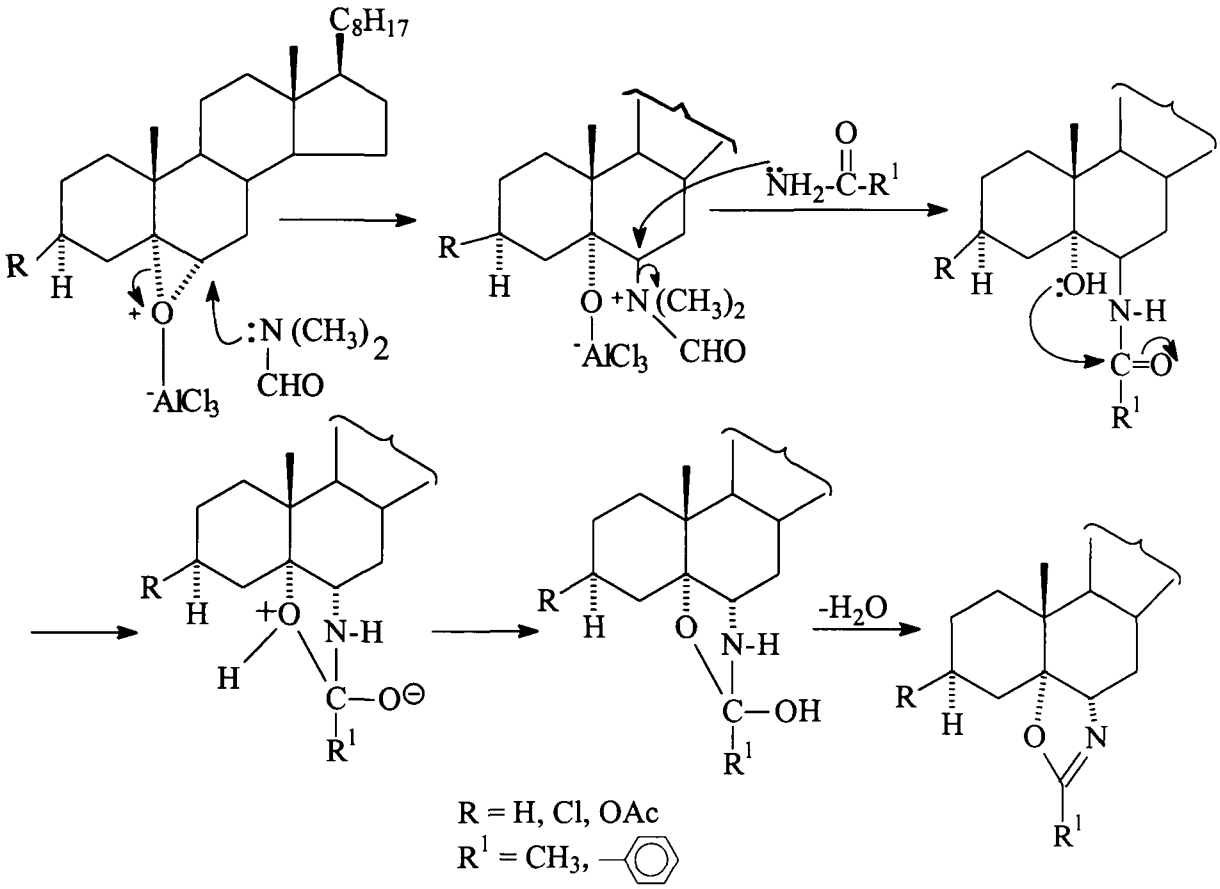
(LXIII-c)
m/z 547 (M^+)

m/z 530

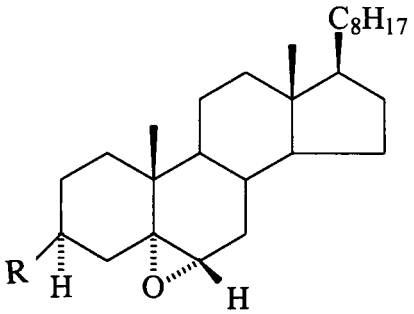
Characterization of compound, m.p. 209 as 3β-acetoxy-5, 6β-dihydroxy-5α-cholestane (XIII-c) :

The compound, m.p. 209° is characterized as 3β-acetoxy-5, 6β-dihydroxy-5α-cholestane (XIII-c) by comparison of analytical and spectral (m.p., m.m.p., TLC, IR and ^{1}H-NMR) data which were found identical with (XIII-c)[3].

A tentative mechanism is written to explain the formation of (LXIII – LXIV) in which a double SN^2-inversion on epoxide ring at C-6 takes place[30].



Several aminosterols and their derivatives possess valuable pharmaceutical properties[25-29] such as tranquillizer, anesthetic, antiarrythmic and sedative. A number of synthetic routes to synthesize these compounds have been developed by different workers[14,15,16,17]. An attempt was made to synthesize these compounds by treating some of the steroidal epoxides in cholestane series with semicarbazide, phenylsemicarbazide and semithiocarbazide. The epoxides studied are 5, 6α-epoxy-5α-cholestane (XII-a), 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) and 3β-acetoxy 5, 6α-epoxy-5α-cholestane (XII-c). The products obtained were characterized on the basis of analytical and spectral (IR, ^{1}H-NMR, ^{13}C-NMR and Mass) evidences.

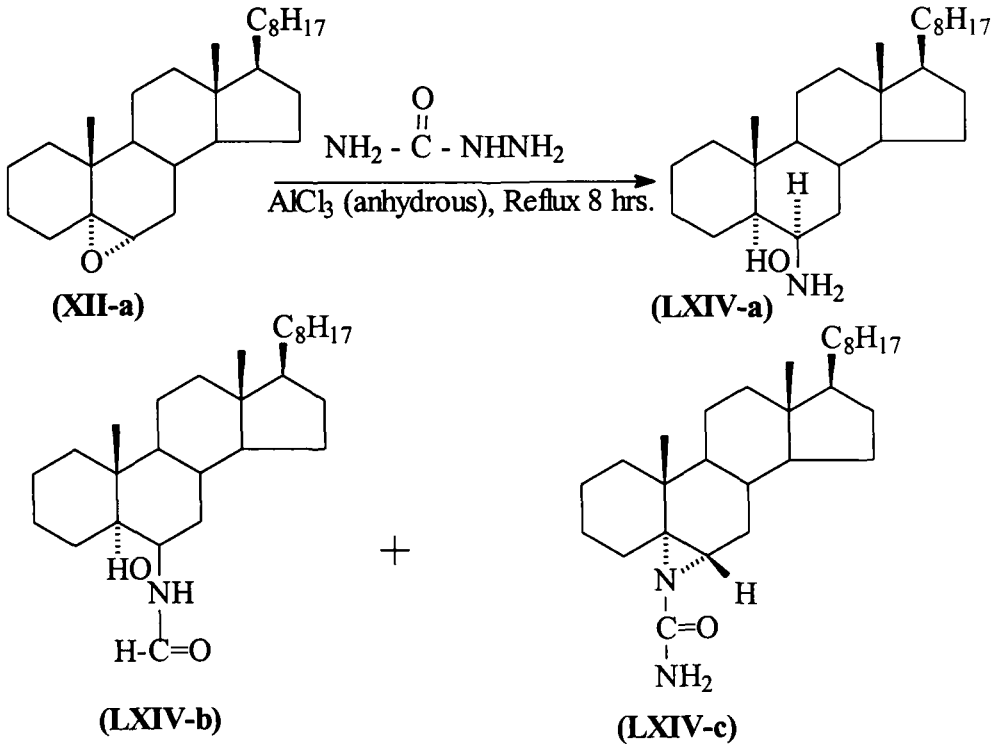


R

(XII-a) H
(XIII-b) Cl
(XII-c) OAc

Reaction of 5, 6α-epoxy-5α-cholestane (XII-a) with semicarbazide : 5-Hydroxy-6β-amino-5α-cholestane (LXIV-a), 5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXIV-b) and N-amido-5α-cholestano [5, 6-b]-aziridine (LXIV-c).

5, 6α-Epoxy-5α-cholestane (XII-a) was treated with semicarbazide in dimethylformamide (anhydrous $AlCl_3$ as catalyst) and the reaction mixture was heated under reflux for 8 hours. After the completion of reaction, the reaction mixture was worked up in usual manner and chromatographed over silica gel to provide three compounds, m.p. 137°, 121° and 115°.



Characterization of the compound, m.p. 137° as 5-hydroxy-6β-amino-5α-cholestane (LXIV-a) :

The mass spectrum of the compound, m.p. 137° showed molecular ion peak at m/z 403 and was analysed correctly for $C_{27}H_{49}NO$. The IR spectrum of the compound showed strong absorption band around 3480 cm^{-1} which was assigned to –OH and –NH groups and 1405 (C-N), 1280, 1040 cm^{-1} (C-O). The ^{1}H-NMR spectrum of the compound exhibited a peak at δ 4.5 (brs) integrating for two protons which could be assigned to $-NH_2$. A multiplet at δ3.75 for one proton is assigned to H-6α. An other broad singlet was seen at δ4.0 was due to hydroxy proton which disappeared on D_20 addition. The other signals were seen at δ 1.02 (C10-CH_3), δ 0.65 (C13-CH_3), and 0.85, 0.77 (side chain methyl protons). The above spectral and elemental analysis suggested the structure of the compound as 5-hydroxy-6β-amino-5α-cholestane (LXIV-a) which was further supported by mass spectral studies. Mass spectrum of compound (LXIV-a) gave molecular ion peak at m/z 403 followed by some significant fragment ions at m/z 388 ($M^{+\bullet}$ -CH_3), m/z 385 ($M^{+\bullet}$ -H_2O), m/z 290 ($M^{+\bullet}$ -C_8H_{17}) and lower mass peaks.

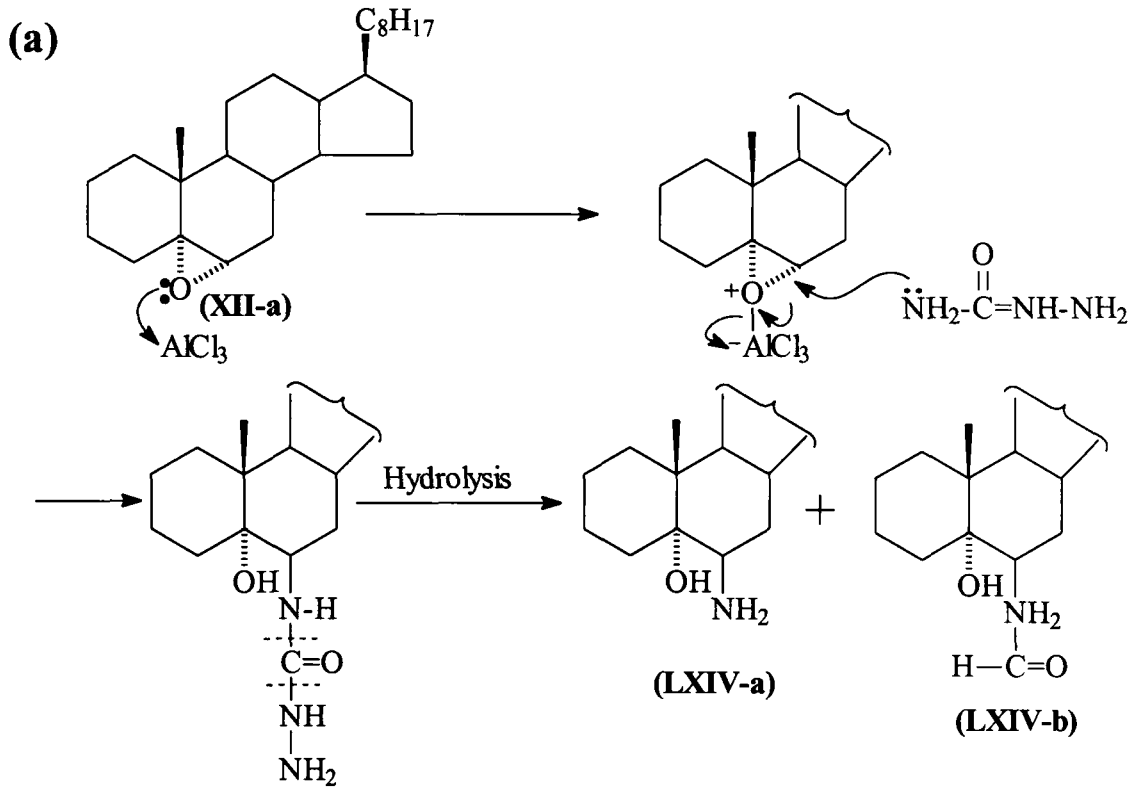
Characterization of compound, m.p. 121° as 5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXIV-b) :

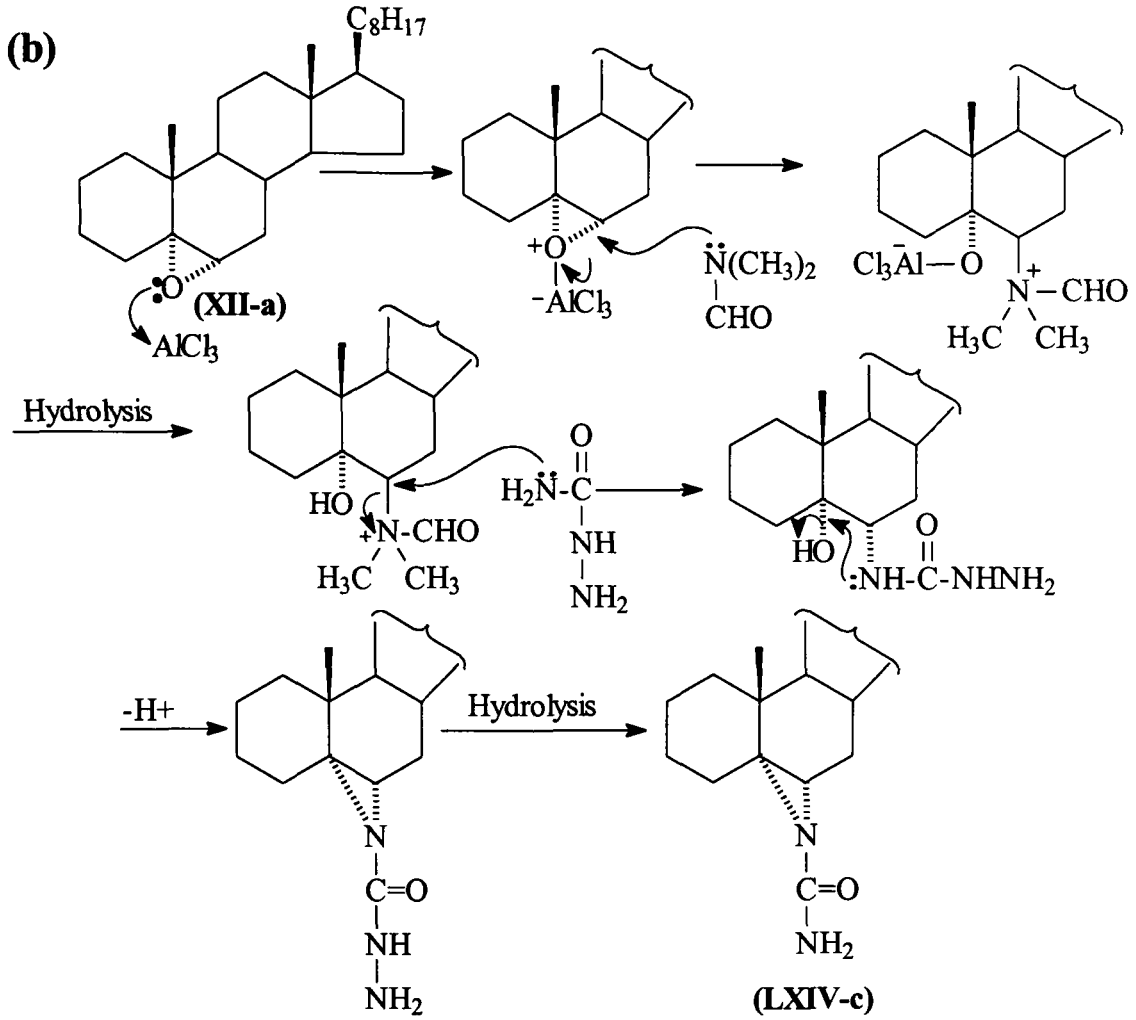
The compound, m.p. 121° was analysed correctly for $C_{28}H_{49}NO_2$. The IR spectrum of the compound showed strong absorption bands at 3510, and 3485 (-NH, -O-H), 1690 (H-C=O) and 1390 cm^{-1}(C-N), ^{1}H-NMR spectrum of the compound showed peaks at δ8.5 (1H, H-C=O), 5.75 (brs, 1H, NH, exchangeable with D_20), 3.5 (mc, 1H, H-6α, equatorial)[33], 2.30 (brs, 1H, exchangeable with D_20, OH), 1.1 (C10 -CH_3), 0.75 (C13-CH_3), 0.92 and 0.83 (side chain methyl protons), on the basis of above spectral evidences, the structure of the compound was confirmed as 5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXIV-b). The structure (LXIV-b) was further supported by mass spectral study. The mass spectrum of the compound (LXIV-b) gave molecular ion peaks at m/z 431 (M+) followed by some significant fragment ions at m/z 416 (M^+ -CH_3), m/z 413 (M^+ -H_2O), m/z 403 (M^+ -CO) and lower mass peaks.

Characterization of the compound m.p. 115° as N-amido-5α-cholestano [5, 6-b]-aziridine (LXIV-c) :

The mass spectrum of the compound, m.p. 115 °C showed molecular ion peak at m/z 385 and was analysed correctly for $C_{28}H_{48}N_2O$. The IR spectrum of the compound showed strong absorption band at 3450 (-N-H), 1685 ($-CONH_2$) and 1390 cm^{-1} (C-N). The ^{1}H-NMR spectrum of the compound exhibited a multiplet at δ 3.1 integrated for one proton which could be assigned to H-6β. A broad singlet was observed at δ 3.1 which was assigned to N–H proton, The other signals were seen at δ1.01 (C10-CH_3), 0.65 (C13-CH_3) and δ0.92, 0.85 (side chain methyl protons). The above spectral and elemental analysis suggested the structure of the compound as 5α-cholestano [5, 6-b]-aziridine (LXIV-c). Mass spectral study further supported the structure of compound as (LXV). The mass spectrum gave molecular ion at m/z 428 ($M^{+\bullet}$), followed by other significant fragment ion peaks at m/z 427 (m/z 428 - H), m/z 413 ($M^{+\bullet}$ - CH_3), m/z 315 ($M^{+\bullet}$ -C_8H_{17}). The formation of these compounds (LXIV) and may be explained by proposed mechanism as given which involved trans diaxial ring opening[14] of epoxide (XII-a). Semicarbazide was used as nucleophile catalysed by anhydrous $AlCl_3$

followed by hydrolysis (path a). The aziridine (LXIV-c) was formed by double SN^2 inversion on the epoxide ring at C6 (path b)[30].

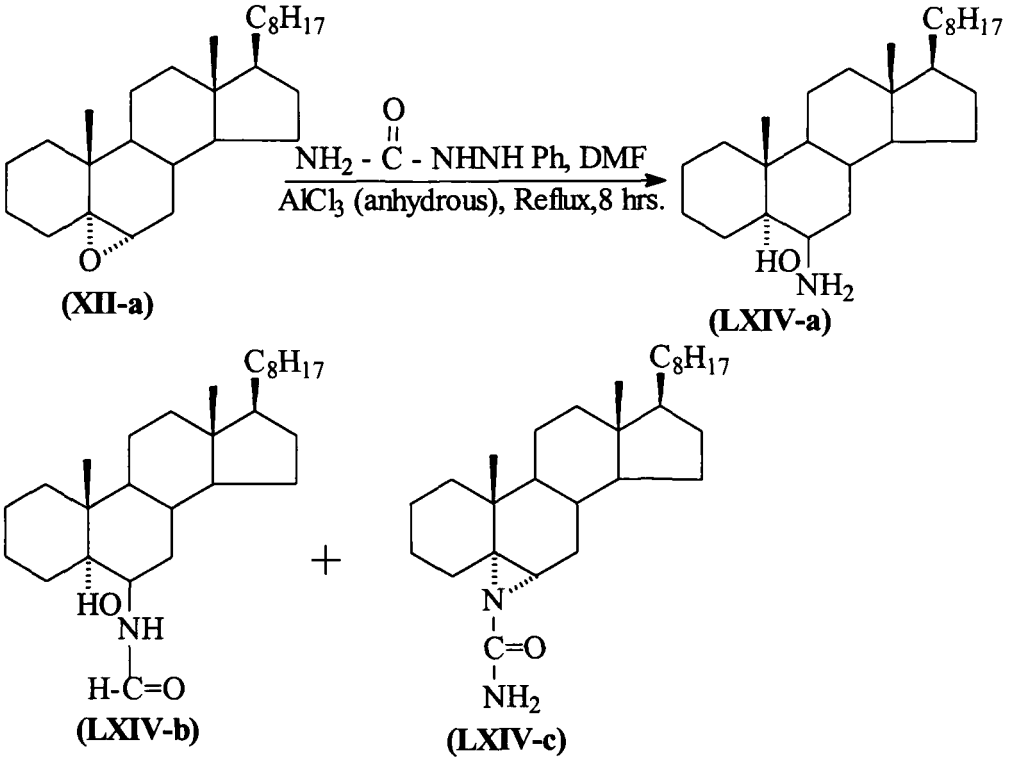




Reaction of 5, 6α-epoxy-5α-cholestano (XII - a) with phenyl semicarbazide : 5-Hydroxy-6β-amino-5α-cholestane (LXIV-a), 5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXIV-b) and N-amido-5α-cholestano [5, 6-b]-aziridine (LXIV-c).

5, 6α-Epoxy-5α-cholestane (XII-a) was treated with phenyl semi-carbazide in DMF (anhydrous $AlCl_3$ as catalyst) and the reaction mixture was

heated under reflux for 8 hrs. After the completion of reaction, the reaction mixture was worked up in usual manner and chromatographed over silica gel to provide three compounds, m.p. 137°, 121° and 115°.



(XII-a) **(LXIV-a)** **(LXIV-b)** **(LXIV-c)**

Characterization of the compound, m.p. 137° as 5-hydroxy-6β-amino-5α-cholestane (LXIV - a) :

The compound, m.p. 137° was correctly analysed for $C_{27}H_{49}NO$. The m.p., m.m.p., TLC, IR, ^{1}H-NMR and mass spectral data were found same and identical with the hydroxy amino compound (LXIV - a) which was obtained

when 5, 6α-epoxy-5α-cholestane (XII-a) was treated under identical reaction conditions with semi carbazide.

Characterization of compound, m.p. 121° as 5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXIV-b) :

The compound, m.p. 121° was correctly analysed for $C_{28}H_4NO_2$. The m.p., m.m.p., TLC, IR ^{1}H-NMR and mass spectral data were same and identical with amino formyl compound (LXIV-b) obtained when 5, 6α-epoxy-5α-cholestane (XII-a) was reacted under identical reaction conditions with semicarbazide.

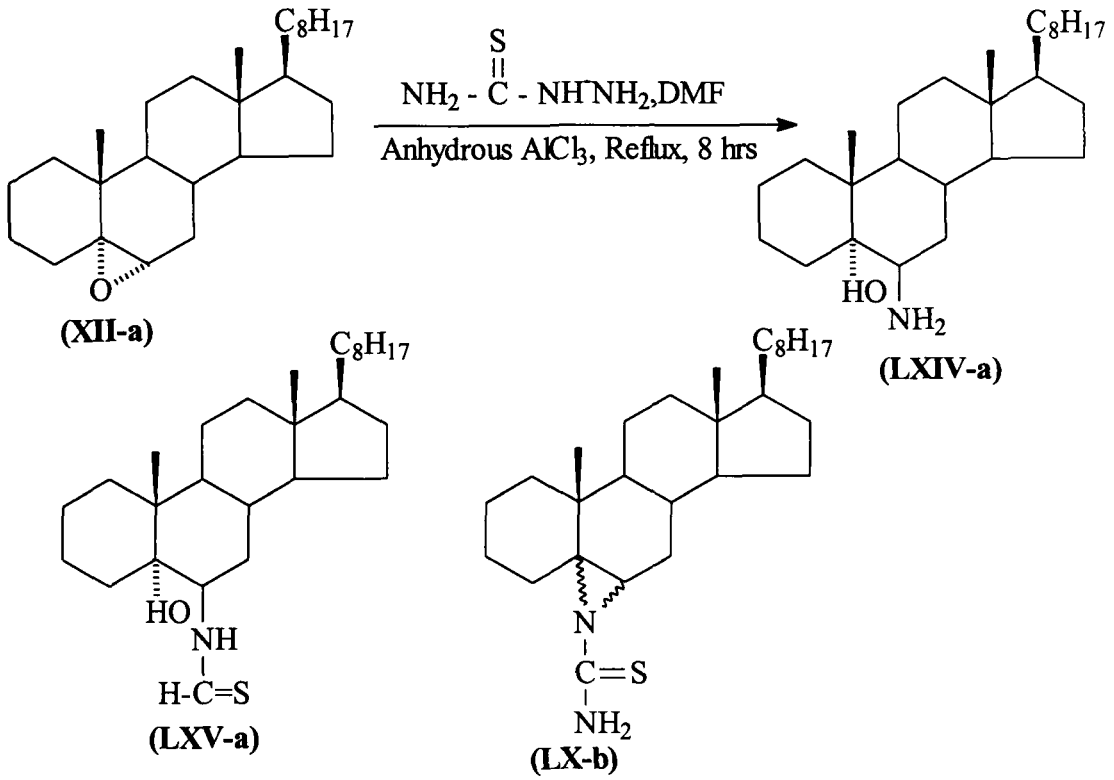
Characterization of the compound, m.p. 115° as N-amido-5α-cholestano [5, 6-d] aziridine (LXIV-c) :

The compound m.p. 115° was analysed correctly for $C_{28}H_{48}N_2$. The m.p., m.m.p., TLC, IR, ^{1}H-NMR and mass spectral data were same and identical with the aziridine (LXIV-c) obtained when 5, 6α-epoxy-5α-cholestane (XII-a) was treated with semicarbazide under identical reaction conditions.

Reaction of 5, 6α-epoxy-5α-cholestane (XII-a) with semi-thiocarbazide : 5-Hydroxy-6β-amino-5α-cholestane (LXIV-a),

5-hydroxy-6β-amino-N_thioformyl-5α-cholestane (LXV-a) and N-thioamido-5α-cholestano [5, 6-b]-aziridine (LXV-b).

5,6α-Epoxy-5α-cholestane (XII-a) was treated with semithiocarbazide in DMF (anhydrous $AlCl_3$ as catalyst) and the reaction mixture was heated under reflux for 8 hrs. After the completion of reaction, the reaction mixture was worked up in usual manner and chromatographed over silica gel to provide three compounds, m.p 138°, 198° and 124°.



(XII-a)

(LXIV-a)

(LXV-a)

(LX-b)

Characterization of compound, m.p. 137° as 5-hydroxy-6β-amino-5α-cholestane (LXIV a) :

The compound, m.p. 137° was analysed correctly as $C_{27}H_{49}NO$. The m.p., m.m.p., TLC, IR, ^{1}H-NMR and mass spectral data of hydroxy amino compound (LXIV-a) were same and identical with the hydroxy amino compound (LXIV-a) was obtained when 5, 6α-epoxy-5α-cholestane (XII-a) was treated with semicarbazide. Therefore the structure of the compound, m.p. 137° was confirmed as 5-hydroxy-6β-amino-5α-cholestane (LXIV-a).

Characterization of the compound, m.p. 198° as 5-hydroxy-6β-amino-N-thioformyl-5α-cholestane (LXV-a) :

The compound m.p. 198° was analysed correctly for $C_{28}H_{49}NSO$. The IR spectrum of the compound showed band at 3545 – 3450 (-OH, -NH), 1520 (-C=S)[34], 1360 cm^{-1} (C-N). In ^{1}H-NMR spectrum of compound (LXVIII), peaks were obtained at δ 7.4 (s, 1H, H-C=S), 5.76 (brs, 1H, -NH, exchangeable with deuterium), 3.85 (mc, 1H, W½ = 6 Hz, H-6α, equatorial). Therefore C6-N band is axial (β-oriented)[33]. Hydroxy proton was observed at δ 2.65 (brs, exchangeable with deuterium). Peaks for angular and side chain

methyl were observed at 1.13(C10 – CH_3), 0.65 (C13 – CH_3), 0.96 and 0.86 (side chain methyl protons). On the basis of above observations, the structure of the compound m.p. 198° can be formulated as 5-hydroxy-6-amino-N-thioformyl-5α-cholestane (LXV-a). The structure (LXV-a) was further supported by its mass spectral study. The mass spectrum of the compound (LXV-a) gave molecular ion peak at m/z 447 ($M^{+\cdot}$) followed by other significant fragment ion peaks at m/z 432 ($M^{+\cdot}$ -CH_3), m/z 429 ($M^{+\cdot}$ -H_2O), m/z 403 ($M^{+\cdot}$ -C=S) and lower mass peaks.

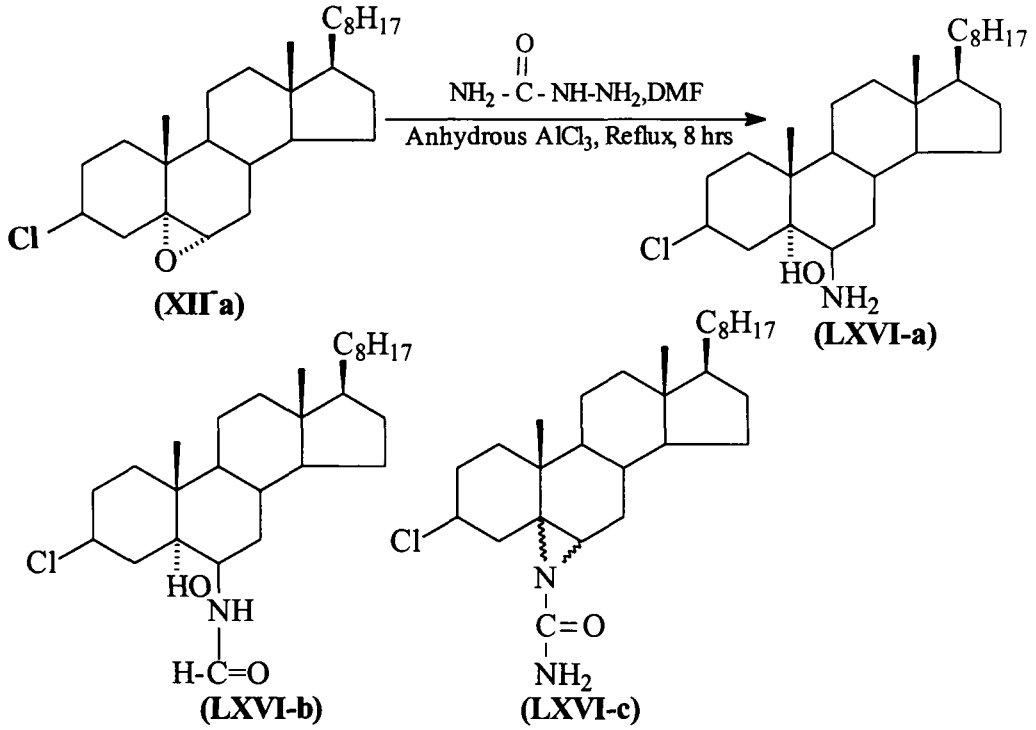
Characterization of the compound, m.p. 124° as N-thioamido-5α-cholestano [5, 6-b] aziridine (LXV-b) :

The compound m.p. 124° was analysed for $C_{28}H_{48}N_2S$. The IR spectrum showed absorption band at 3425 (-NH), 1525 (C=S), 1360 cm^{-1} (C-N). The ^{1}H-NMR spectrum displayed a sharp singlet at δ 5.9 for (NH_2) 3.8 (mc, 1H, W½ = 10.5 Hz) for (H-6β). Other signals were observed at 1.01 (C10-CH_3), 0.65 (C13-CH_3) 0.92, 0.86 (side chain methyl protons). On the basis of above observations, the structure for the compound m.p. 124° can be formulated as N-thioamido-5α-cholestano [5, 6-b]-aziridine (LXV-b). The

structure of aziridine (LXV-b) was further supported by mass spectral evidence. The mass spectrum of the compound (LXV-b) gave molecular ion peak at m/z 444 ($M^{+\bullet}$) followed by other significant fragment ion peaks at m/z 429 ($M^{+\bullet}$ - CH_3), m/z 427 ($M^{+\bullet}$ - NH_3), m/z 400 ($M^{+\bullet}$ -C=S) and lower mass peaks.

Reaction of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) with semicarbazide : 3β-Chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a), 3β-Chloro-5-hydroxy-6β-amino-N-formyl-5α-cholest-ane (LXVI-b) and 3β-chloro-N-amido-5α-cholestano [5, 6-b]-aziridine (LXVI-c).

The epoxide (XII-b) dissolved in DMF was treated with semicarbazide (anhydrous $AlCl_3$ as catalyst) under reflux condition for 8 hrs. The reaction mixture, after usual work up and column chromatography over silica gel, afforded three compounds m.p. 173°, 153° and 132°.



Characterization of compound, m.p. 173° as 3β-chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a) :

The compound, m.p. 173° showed molecular ion peaks at m/z 437/439 (M+) in its mass spectrum and was analysed for $C_{27}H_{48}NOCl$ (positive Beilstein test). The IR spectrum of the compound exhibited absorption bands at 3585 and 3450 cm^{-1} for –OH and –NH groups. The other bands were at 1395 (C-N) and 760 cm^{-1} (C-Cl). ^{1}H-NMR spectrum of the compound (LXVI-a) gave peak at δ 4.7 as broad singlet for $-NH_2$ protons which disappeared on D_2O exchange. A multiplet at δ 3.6 was assigned to H-6α

($W½$ = 5.5 Hz)indicating that amino group is axial[33]. The H-3α appeared at δ4.1 as a multiplet ($W½$ = 17 Hz, axial)[33] and hydroxyl proton gave peak as broad singlet at δ 2.35. Methyl signals were seen at δ 1.12 (C10-CH_3), 0.75 (C10-CH_3), 0.93 and 0.87 (side chain methyl protons). Therefore discussion suggested the structure of the above compound as 3β-chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a). Further support for (LXVI-a) was obtained from its mass spectral study. The mass spectrum gave molecular ion peaks at m/z 437/439 ($M^{+\cdot}$) followed by other significant fragment ions peaks at m/z 422/424 ($M^{+\cdot}$ - CH_3), m/z 419/421 ($M^{+\cdot}$ - H_2O), m/z 383 (m/z 419 – HCl), m/z 366 (m/z 383 – NH_3).

Characterization of the compound, m.p.153°as 3β-chloro 5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVI-b) :

The mass spectrum of the compound, m.p. 153° showed molecular ion peak at m/z 465/467 in mass spectrum and was analysed for $C_{28}H_{48}NO_2Cl$ (showed positive Beilstein test). The IR spectrum of the compound exhibited absorption bands at 3590 and 3445 cm^{-1} for-OH and-NH groups. The band at 1695 cm^{-1} indicated the presence of a carbonyl group in the molecule. The

other bands were 1405 (C-N), 730 cm^{-1} (C-Cl). The ^{1}H-NMR spectrum displayed a broad singlet at δ 8.2 for one proton of the formyl group. A multiplet at δ 5.6 which could be assigned to –NH proton at C6. A broad singlet at δ 4.2 was due –OH proton which disappeared on D_2O exchange. The multiplets for H-3α and H- 6α appeared at δ 3.48. The methyl signals were seen at δ 1.2 (C10-CH_3), δ 0.7 (C13-CH_3), δ 0.93 – 0.83 (for side chain methyl protons). The foregoing discussion suggested the structure of the above compound as 3β-chloro-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVI-b). Mass spectral study further supported the above assigned structure for compound (LXVI-b). The mass spectrum of (LXVI-b) gave molecular ion at m/z 465/467 ($M^{+\bullet}$) followed by some significant fragment ion peaks at 450/452 ($M^{+\bullet}$ - CH_3), m/z 447/449 ($M^{+\bullet}$ - H_2O), m/z 429 ($M^{+\bullet}$ - HCl) and lower mass peaks.

Characterization of compound, m.p. 132° as 3β-chloro-N-amido-5α-cholestano [5,6 – b] aziridine (LXVI-c) :

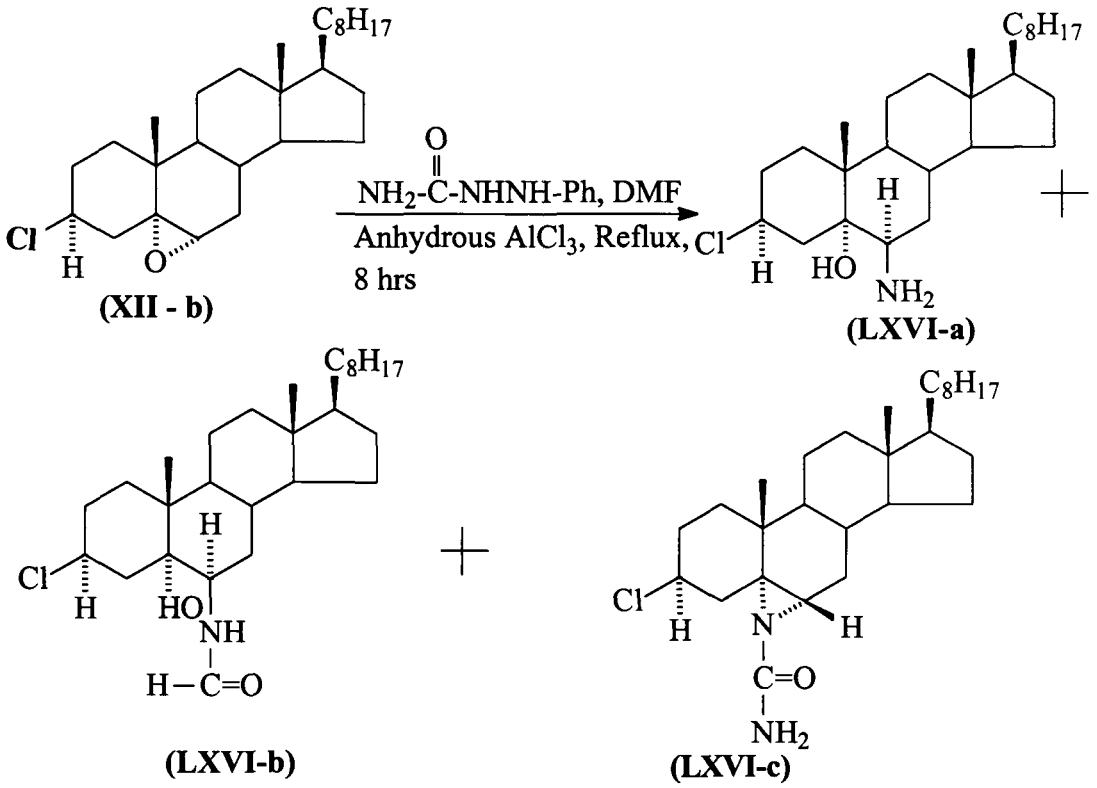
The compound, m.p. 132° was correctly analysed for $C_{28}H_{47}N_2OCl$. (positive Beilstein test). The IR spectrum of the compound exhibited bands at

3460 (-N-H), 1690 (-CO-NH), 1395 (C-N) and 765 cm^{-1} (C-Cl). ^{1}H-NMR spectrum of the compound gave peaks at δ 5.1 (S, 2H, exchangeable with D_2O, $-NH_2$), 3.90 (mc, 1H, W½ = 6.2 Hz, H-6β, axial)[33], 4.3 (mc, 1H, W½ = 18 Hz, H-3α , axial)[33]. The methyl protons gave signals at δ 1.1 (C10-CH_3), 0.70 (C13- CH_3), 0.95 and 0.88 (side chain methyl protons). On the basis of above analytical and spectral evidences, the structure of compound, m.p. 132° was suggested as 3β-chloro-N-amido-5α-cholestano [5, 6–b] aziridine (LXVI-c). The structure (LXVI-c) was further supported by its mass spectral analysis. The mass spectrum of the compound (LXVI-c) gave molecular ion peaks at m/z 462/464 ($M^{+\bullet}$) followed by other important fragment ion peaks at m/z 447/449($M^{+\bullet}$ -CH_3), m/z 445/447 ($M^{+\bullet}$ -NH_3), m/z 426 ($M^{+\bullet}$ -HCl), 418/420 ($M^{+\bullet}$ -NH_2CO) and lower mass fragment ion peaks.

Reaction of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) with phenyl semi carbazide :

The epoxide (XII-b) was treated with phenyl semi carbazide in dimethyl formamide (anhydrous $AlCl_3$ as catalyst) under reflux condition for

8 hrs. The reaction mixture after usual work up and column chromatography over silica gel afforded three compounds m.p. 173°, 151-153° and 132-133°.



Characterization of compound, m.p. 173° as 3β-chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a) :

The compound, m.p. 173° was analysed for $C_{28}H_{48}NOCl$ (positive Beilstein test). The m.p., m.m.p. TLC, IR, ^{1}H-NMR and mass spectral data

were found identical with hydroxyamino compound (LXVI-a) found when 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) was treated with semicarbazide under identical conditions.

Characterization of the compound m.p. 151–153°, as 3β-chloro-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVI-b) :

The compound m.p. 151-153° was analysed correctly for $C_{28}H_{48}NO_2Cl$ and showed positive Beilstein test. The m.p., m.m.p., IR, ^{1}H-NMR and Mass spectral data were same and identical with amino formyl compound (LXVI-b) found when 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) was treated with semicarbazide under identical condition and the structure of the compound m.p. 151 - 153° was confirmed as 3β-chloro-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVI-b).

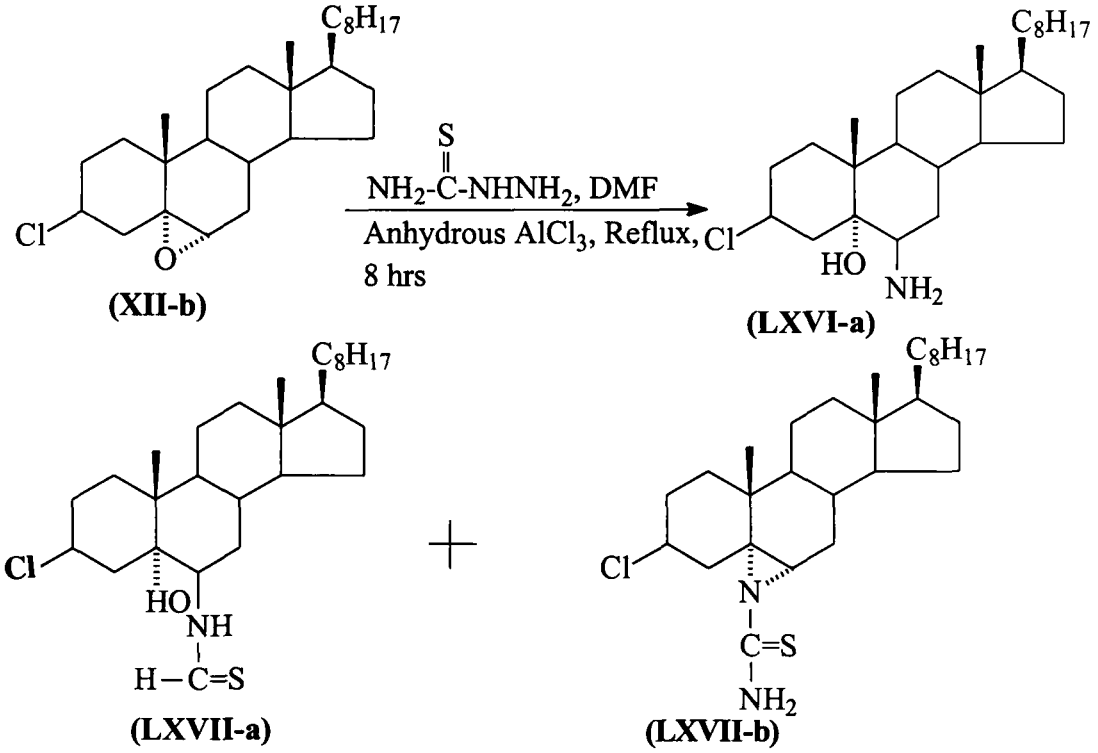
Characterization of the compound as m.p. 131-132° as 3β-chloro-N-amido-5α-cholestano [5, 6-b] aziridine (LXVI-c) :

The compound m.p. 131-132° was correctly analysed for $C_{28}H_{48}N_2OCl$ and gave positive Belistein test. The m.p., m.m.p., TLC, IR, ^{1}H-NMR and Mass spectral data were same and identical with the aziridine found when 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) was treated under identical

conditions with semicarbazide. Therefore the structure of the compound m.p. 131-132° could be assigned as 3β-chloro-N-amido-5α-cholestano [5, 6-b] aziridine (LXVI-c).

Reaction of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) with semithiocarbazide : 3β-Chloro-5-hydroxy-6β-amino-5α-cholestane(LXVI-a), 3β-chloro-5-hydroxy-6β-amino-N-thio-formyl-5α- cholestane (LXVII-a) and 3β-chloro-N-thioamido-5α-cholestano [5,6-b]-aziridine (LXVII-b).

The epoxide (XII-b) was treated with semithiocarbazide in dimethyl formamide (anhydrous $AlCl_3$ catalyst) under reflux condition for 8 hrs. The reaction mixture, after usual work up and column chromatography over silica gel afforded three compounds m.p 172-73°, oil 141-42°.



Characterization of the compound m.p. 172-73° as 3β-chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a) :

The compound m.p. 172-73° was analysed correctly for $C_{27}H_{48}NOCl$ (positive Beilstein test) and found identical (m.p., m.m.p., TLC, IR, ^{1}H-NMR and Mass spectral evidences) with hydroxy amino compound (LXVI-a) found when 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) was treated under identical conditions with semicarbazide or phenyl semicarbazide.

Characterization of oily compound as 3β-chloro-5-hydroxy-6β-amino-N-thioformyl-5α-cholestane (LXVII-a) :

The oily compound was analysed correctly for $C_{28}H_{48}ONSCl$ (positive Beilstein test). The IR spectrum of the compound showed bands at 3560 - 3435 (-OH, -NH), 1530 (H-C=S), 1390 (C-N) and 740 cm^{-1} (C-Cl), ^{1}H-NMR spectrum of the compound displayed a sharp singlet at δ 8.1 for one proton of formyl group. A broad singlet for one proton was seen at δ 5.35 (exchangeable with deuterium) was assigned to –NH proton. A multiplet at δ 4.30 was assigned to H-3α (W½ = 16 Hz, axial)[33], another multiplet at δ 3.75 for H-6α (W½ = 6 Hz) and a broad singlet at δ 2.25 for C5-hydroxy proton (exchangeable with deuterium) were also observed in ^{1}H-NMR spectrum. Angular and side chain methyl protons were observed at δ 1.15 (C10-CH_3), 0.70 (C13-CH_3), 0.95 and 0.85 (side chain methyl protons). On the basis of above evidences, the compound (LXVII-a) was identified as 3β-chloro-5-hydroxy-6β-amino-N-thioformyl-5α-cholestane (LXVII-a). Further support for this structure was obtained by its mass spectral study. The mass spectrum of the compound (LXVII-a) gave molecular ion peaks at m/z 481/483 ($M^{+\bullet}$)

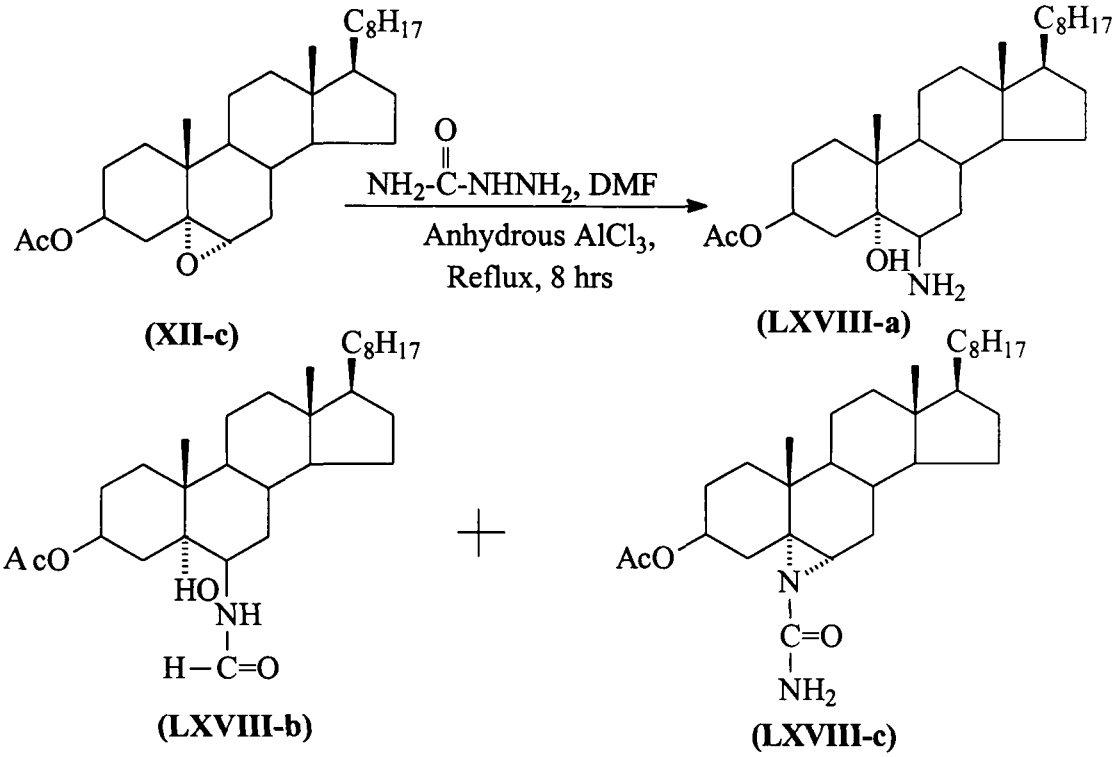
followed by some significant fragment ion peaks at m/z 466/468 ($M^{+\bullet}$ -CH_3), m/z 463/465 ($M^{+\bullet}$ -H_2O),m /z 445 ($M^{+\bullet}$ -HCl) and lower mass peaks.

Characterization of compound, m.p. 141-42° as 3β-chloro-N-thioamido-5α-cholestano [5, 6-b]- aziridine (LXVII-b) :

The compound, m.p. 141-42° was analysed as $C_{28}H_{47}N_2SCl$ (positive Beilstein test). The IR spectrum of the compound exhibited bands at 3435 (-NH), 1530 (C=S), 1310 (C-N) and 765 cm^{-1} (C-Cl). In ^{1}H-NMR spectrum peaks were found at δ 5.4 (s, 2H, -NH_2C=S), 3.38 (mc, 1H, W½ = 6.5 Hz, H-6β, axial)[33], 3.55 (mc, 1H, W½ = 17 Hz, H-3α, axial), 1.15 (C10-CH_3), 0.73 (C13-CH_3), 0.93 and 0.88 (side chain methyl protons). The structure (LXVII-b) was further supported by its mass spectral study. The mass spectrum of the compound gave molecular ion peaks at m/z 478/480 ($M^{+\bullet}$) followed by some significant fragment ion peaks at m/z 463/465 ($M^{+\bullet}$ -CH_3), m/z 461/463 ($M^{+\bullet}$ -NH_3), m/z 434/436 ($M^{+\bullet}$ -NH_2CO), m/z 442 ($M^{+\bullet}$ -HCl) and lower mass peaks.

Reaction of 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) with semicarbazide : 3β-Acetoxy-5-hdroxy-6β-amino-5α-cholestane (LXVIII-a), 3β-acetoxy-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVIII-b) and 3β-acetoxy-N-amido-5α-cholestano [5, 6-b]-aziridine (LXVIII-c).

The epoxide (XII-c) was heated under reflux with semicarbazide (anhydrous $AlCl_3$ as catalyst) in dimethyl formamide for 8 hrs. The usual work up of the reaction mixture and column chromatography over silica gel provided three compounds m.p 225-227°, 201-203° and 177°-178°.



(XII-c) **(LXVIII-a)** **(LXVIII-b)** **(LXVIII-c)**

Characterization of the compound m.p 225-227° as 3β-acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a) :

The mass spectrum of the compound, m.p 225 – 227° was analysed for $C_{29}H_{51}NO_3$. The IR spectrum of the compound exhibited bands at 3530 - 3485 (-NH, OH), 1730 (-$COCH_3$), 1285, 1045 (C-O), 1365 cm^{-1} (C-N). In ^{1}H-NMR peaks were observed at δ 5.15 (mc, 1H, W½ = 18 Hz, axial, H-3α)[33], 4.50 (brs, 2H, -NH_2) 3.1 (mc, 1H, W½ = 6.8 Hz, H-6α), 2.75 (brs, 1H, exchangeable with D_2O, -OH), 2.08 (s, 3H, -O-CO-CH_3), 1.15 (C10-CH_3), 0.68 (C13-CH_3), 0.97 and 0.85 (side chain methyl protons). Further support of the structure (LXVIII-a) was given by mass spectral study. The mass spectrum of (LXVIII-a) gave molecular ion peak at m/z 461 ($M^{+\bullet}$) followed by other significant fragment ion peaks at m/z 444 ($M^{+\bullet}$ -NH_3), m/z 443 ($M^{+\bullet}$ -H_2O), m/z 446 ($M^{+\bullet}$ -CH_3) and lower mass peaks.

Characterization of compound, m.p. 201-203° as 3β-acetoxy-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVIII-b) :

The compound, m.p. 201-203° was analysed correctly for $C_{30}H_{51}NO_4$. The IR spectrum of the compound showed absorption bands at 3510 – 3450

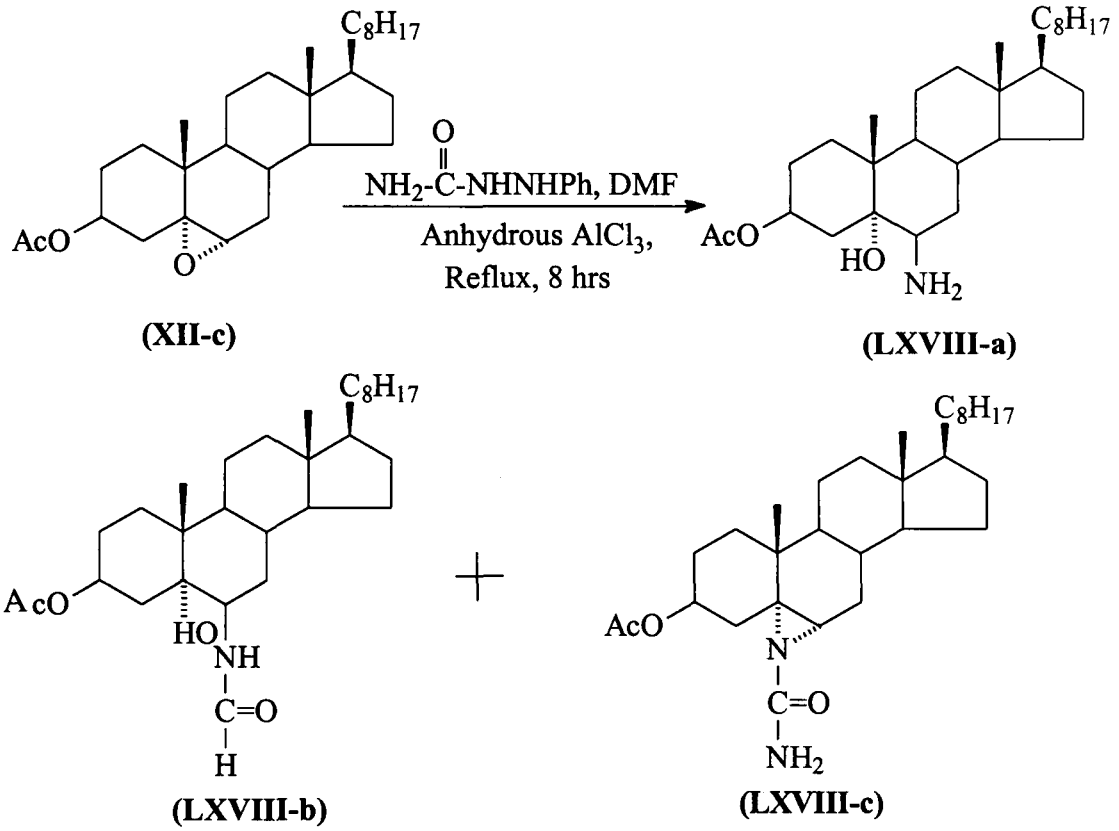
(-NH, OH), 1725 (-CO-CH_3), 1330 (C-N), 1280, 1050 cm^{-1} (C-O). ^{1}H-NMR spectrum of the compound exhibited a sharp singlet at δ 8.2 for the formyl proton and a multiplet at δ 5.1 for H-3α (W½ = 18 Hz), another multiplet at δ5.5 was assigned to –NH proton (exchangeable with deuterium). The H-6α was exhibited as multiplet at δ 3.8 (W½ = 6 Hz), suggesting the amino group as axial[33]. A singlet at δ 2.1 was ascribed for acetate methyl protons. Methyl signals were present at δ 1.13 (C10-CH_3), 0.72 (C13-CH_3), 0.91 and 0.88 for side chain methyl protons. On the basis of above evidences, the structure of compound m.p. 201-203° was assigned to 3β-acetoxy-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVIII-b). Further support for structure (LXVIII-b) was obtained from mass spectral study. The mass spectrum gave molecular ion peak at m/z 489 ($M^{+\bullet}$) followed by some significant fragment ion peaks at m/z 474 ($M^{+\bullet}$ -CH_3), m/z 471 ($M^{+\bullet}$ -H_2O), m/z 429 ($M^{+\bullet}$ -CH_3COOH) and lower mass peaks.

Characterization of compound, m.p. 177-178° as 3β-acetoxy-N-amido-5α-cholestano [5, 6-d] aziridine (LXVIII-c) :

The compound, m.p. 177-178° was analysed correctly for $C_{30}H_{50}N_2O_3$. The IR spectrum of the compound showed absorption bands at 3470 (-NH), 1735 (-CO-CH_3), 1695 (NH-CO-), 1390 (C-N), 1240, 1040 cm^{-1} (C-O). ^{1}H-NMR spectrum of the compound gave peaks at δ 5.20 (s, 2H, exchangeable with D_2O, -NH_2), 5.05 (mc, 1H, W½ = 18 Hz, H-3α, axial)[33]. 4.05 (mc, 1H, W½ = 11 Hz, H-6β)[33], 2.3 (s, 3H, acetate methyl protons), 1.1 (C10-CH_3) 0.74 (C13-CH_3), 0.97 and 0.85 (side chain methyl protons). On the basis of above analytical and spectral evidences, the structure of compound, m.p. 177-178° was established as 3β-acetoxy-N-amido-5α-cholestano [5, 6-b] aziridine (LXVIII-c). Mass spectral study also supported the structure (LXVIII-c). The mass spectrum of the compound gave molecular ion at m/z 486 ($M^{+\bullet}$) followed by some significant fragment peaks m/z 471 ($M^{+\bullet}$ -CH_3), m/z 442 ($M^{+\bullet}$ -NH_2CO), m/z 426 ($M^{+\bullet}$ -CH_3COOH) and lower fragment ion mass peaks.

Reaction of 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) with phenylsemicarbazide : 3β-Acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a), 3β-acetoxy-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVIII-b) and 3β-acetoxy-N-amido-5α-cholestano [5, 6-b] aziridine (LXVIII-c).

The epoxide (XII-c) was heated under reflux with phenyl semicarbazide (anhydrous $AlCl_3$ as catalyst) in dimethyl formamide for 8 hrs. The usual workup of the reaction mixture and column chromatography over silica gel provided three compounds m.p. 226 - 227°,201-202° and 177-179°.



(XII-c) **(LXVIII-a)**

(LXVIII-b) **(LXVIII-c)**

Characterization of compound, m.p. 226-227° as 3β-acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a) :

The compound, m.p. 226-227° was analysed correctly for $C_{29}H_{51}NO_3$. The m.p., m.m.p., IR, ^{1}H-NMR and Mass spectral data were found same and identical with the hydroxy amino compound (LXVIII-a) found when 3β-

acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) was treated with semicarbazide under identical conditions.

Characterization of the compound,m.p. 201-202° as 3β-acetoxy-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVIII-b):

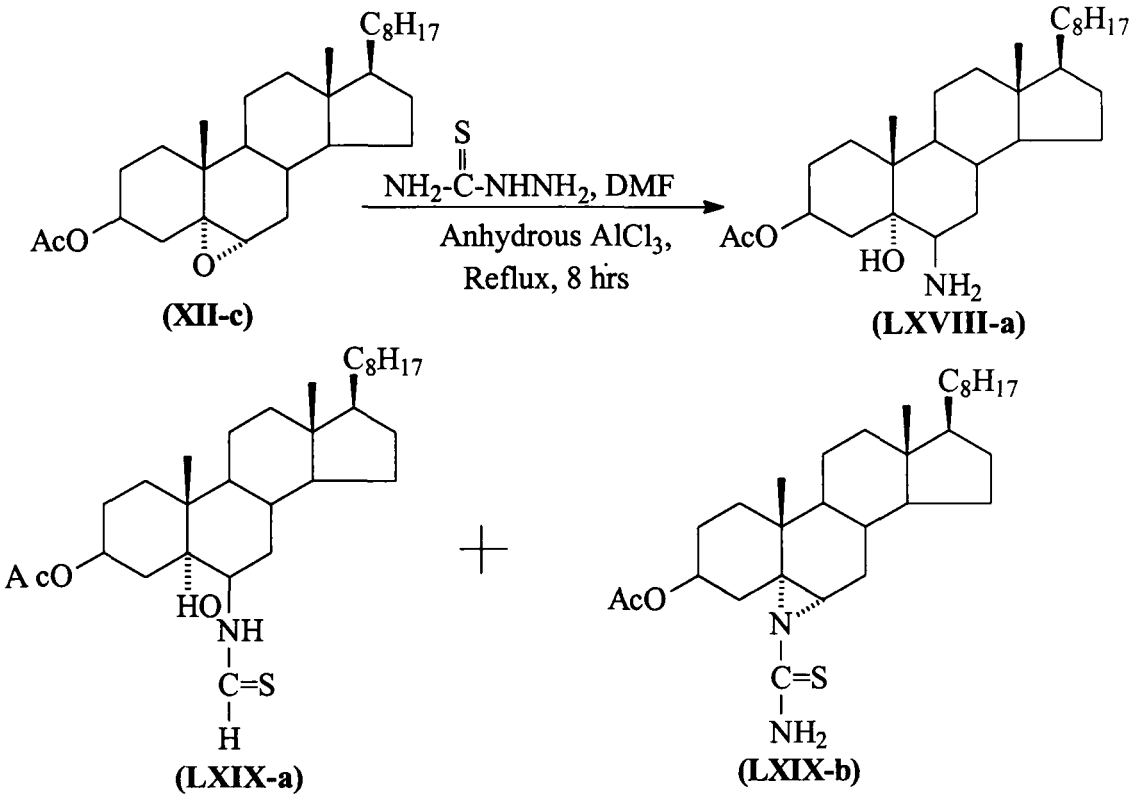
The compound, m.p. 201-202° was analysed correctly for $C_{30}H_{51}NO_4$. The m.p., m.m.p., IR, ^{1}H-NMR and Mass spectral data were same and identical with the amino formyl compound (LXVIII-b) obtained when 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-C) was treated under similar reaction conditions with semicarbazide.

Characterization of compound, m.p. 177-178° as 3β-acetoxy-N-amido-5α-cholestano [5, 6-b] aziridine (LXVIII-c) :

The compound, m.p. 177-178° was analysed correctly for $C_{30}H_{50}N_2O_3$. The m.p., m.m.p., TLC, IR, ^{1}H-NMR and Mass spectral data were found same and identical with thioaziridine (LXVIII-c) found when 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) was treated with semicarbazide under same reaction conditions.

Reaction of 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) with semithiocarbazide : 3β-Acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a), 3β-acetoxy-5-hydroxy-6β-amino-N-thioformyl-5α-cholestane (LXIX-a) and 3β-acetoxy-N-thioamido-5α-cholestano [5, 6-b]-aziridine (LXIX-b).

The 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) was treated with semithiocarbazide (anhydrous $AlCl_3$ as catalyst) in DMF and the reaction mixture was heated under reflux for 8 hrs. The usual workup of the reaction mixture and column chromatography over silica gel provided three compounds, m.p. 225-226°, 230° and 163-164°.



(XII-c) **(LXVIII-a)**

(LXIX-a) **(LXIX-b)**

Characterization of compound, m.p. 225-226° as 3β-acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a) :

The compound, m.p. 225-226° was analysed correctly for $C_{29}H_{51}NO_3$. The m.p., m.m.p., TLC, IR, ^{1}H-NMR and Mass spectral data was found same and identical with the hydroxy amino compound (LXVIII-a) found when 3β-acetoxy-5, 6α-epoxy-5α-cholestane(XII-c) was treated under identical reaction conditions with semicarbazide and phenyl semi carbazide.

Characterization of compound, m.p. 230° as 3β-acetoxy-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXIX-a) :

The compound, m.p. 230° was correctly analysed for $C_{30}H_{51}O_3NS$. In its IR spectrum, absorption bands were observed at 3510, 3485 (-OH, -NH), 1735 (-CO-CH_3), 1525 (C=S), 1370 (C-N), 1250, 1030 cm^{-1} (C-O). ^{1}H-NMR spectrum of the compound gave peaks at δ 8.2 (H-C=S), 5.24 (brs, 1H, -NH, exchangeable with D_2O), 5.1 (mc. 1H, W½ = 18 Hz, H-3α, axial), 3.26 (mc, 1H, W½ = 6 Hz, H-6α), 2.01 (s, 3H, -O-CO-CH_3), 2.27 (brs, 1H, exchangeable with D_2O, -OH), 1.15 (C10-CH_3), 0.68 (C13-CH_3), 0.93 and 0.87 (side chain methyl protons). On the basis of the evidences, the compound, m.p. 230° was identified as 3β-acetoxy-5-hydroxy-6-amino-N-formyl-5α-cholestane (LXIX-a). Further support for the structure (LXIX-a) was obtained from mass spectral study. The mass spectrum of (LXIX-a) gave molecular ion peaks at m/z 505 ($M^{+\bullet}$) followed by some significant fragment ion peaks at m/z 490 ($M^{+\bullet}$ -CH_3), m/z 487 ($M^{+\bullet}$ -H_2O) m/z 445 ($M^{+\bullet}$ - CH_3COOH) and lower mass peaks.

Characterization of compound, m.p. 163-164° as 3β-acetoxy-N-thioamido-5α-cholestano [5, 6-b] aziridine (LXIX-b) :

The compound m.p 163-164° was analysed correctly for $C_{30}H_{50}N_2SO_2$. The IR spectrum of the compound showed absorption bands at 3440 (-NH), 1735 ($COCH_3$), 1525 (C=S), 1380 (CN), 1240, 1025 cm^{-1} (C-O). ^{1}H-NMR spectrum of the compound gave peaks at δ 5.4 (s, 2H, $-NH_2C=S$), 5.1 (mc, 1H, W½ = 17.5 Hz, H-3α, axial)[33], 3.16 (mc, 1H, W½ = 11 Hz, H-6β, axial), 2.2 (s, 3H, acetate methyl protons), δ1.2 (C10-CH_3), δ0.73 (C13-CH_3), 0.96 and 0.85 (side chain methyl protons). On the basis of above evidence, the structure of the compound was assigned as 3β-acetoxy-N-thioamido-5α-cholestano [5, 6-b] aziridine (LXIX-b). Mass spectral study of the compound gave further support to structure (LXIX-b). The mass spectrum gave molecular ion peak at m/z 502 ($M^{+\bullet}$) followed by some significant fragment ion peaks at m/z 487 ($M^{+\bullet}$ -CH_3), m/z 485 ($M^{+\bullet}$ -NH_3), m/z 458 ($M^{+\bullet}$ -C=S) and lower mass peaks.

EXPERIMENTAL

All melting points were observed on a Kofler hot block apparatus and are uncorrected. IR spectra were obtained in KBr/Nujol with a Pye – Unicam SP3 – 100 spectrophotometer and JASCO A – 100 spectrometer. IR values are given in cm^{-1}. ^{1}H-NMR spectra were run in $CDCl_3$ on a Varian A – 60D and XL – 40C spectrometer with Me_4Si as the internal standard and its values are given in ppm (δ) (s = singlet, d= doublet, t = triplet, dd = double doublet, br = broad and mc = multiplet centred at). Mass spectra were measure on JMS D-300/AIE MS – 9 and JEOL JMS – DX 300 spectrometer. Thin layer chromatographic (TLC) plates were coated with silica gel and sprayed with 20% aqueous solution of perchloric acid. Silica gel (20 g) was used for one gm of the material to be separated in column chromatography. Petroleum ether refers to a fraction of b.p. 40 – 60°, sodium sulphate (anhydrous) was used as the drying agent.

3β-Chlorocholest-5-ene (LXX) :

Freshly purified thionyl chloride (75 ml) was added gradually to cholesterol (100 g) at room temperature. A vigorous reaction ensured with the evolution of gaseous products. When the reaction slackened, the reaction

mixture was gently heated at a temperature of 50 - 60° on a water bath for 1hr. and then poured on to crushed ice-water with stirring. The yellow solid thus obtained was filtered under suction and washed several times with ice-water and air dried. Recrystallization of crude product from acetone gave compound (LXX) (95.5 g), m.p. 95 - 96° (reported[35], m.p. 96°). It gave positive Beilstein test and a yellow colour with tetra nitromethane in chloroform.

Cholest-5-ene (LXXI).

3β-Chlorocholest-5-ene (LXX) (15.0 g) was dissolved in warm amyl alcohol (300 ml) and sodium metal (35.0 g) was added in small portions to the solution with continuous stirring over a period of 8 hrs. The reaction mixture was heated now and then during the course of reaction in order to keep the sodium metal dissolved. The reaction mixture was poured into water, acidified with HCL and allowed to stand overnight. A white crystalline solid was obtained which was filtered under suction and washed thoroughly with water and dried. Recrystallization of crude material from acetone gave compound (LXXI) in cubes (10.8 g), m.p. 93° (reported[36], m.p. 89.5 – 91.5°).

5, 6α-Epoxy-5α-cholestane (XII – a) :

Cholest-5-ene (LXXI) (6 g) in chloroform (40 ml) was treated with a solution of perbenzoic acid (1.1 mol equivalent) in chloroform and left at -8° for 20 hrs. The progress of the reaction is monitored by TLC. After the completion of the reaction mixture was washed successively with ice – cold sodium bicarbonate solution (5%), water and sodium thiosulphate solution (5%) and then water and dried over sodium sulphate (anhydrous). Evaporation of the solvent yielded (XII-a) as an oil which was crystallized from acetone as needles (4.3 g), m.p. 76° (reported[32], m.p. 76°).

3β-Chloro-5, 6α-epoxy-5α-cholestane (XII b) :

3β-Chlorocholest-5-ene (LXX) (11 g) in chloroform (100 ml) was treated with a solution of perbenzoic acid (1.1 mol equivalent) and left for 20 hrs. at a temperature of - 8°. After the completion of reaction, the reaction mixture was washed successively with ice – cold sodium bicarbonate solution (5%), water, sodium thiosulphate (5%) solution and again with water. Evaporation of the solvent yielded (XII-b) as an oil which was crystallized from acetone as needles (8.1 g), m.p. 89° (reported[37], m.p. 89.5 – 90.5°).

3β-Acetoxycholest-5-ene (LXXII).

A mixture of cholesterol (50.0 g), pyridine (75 ml, freshly distilled over KOH) and freshly distilled acetic anhydride (50 ml) was heated on steam bath for 2 hrs. The resulting brown solution was poured on to crushed ice-water mixture with stirring. A light brown solid was obtained which was filtered under suction, washed with water to remove pyridine and air dried. The crude product on recrystallization from acetone gave pure 3β-acetoxycholest-5-ene (LXXII) (45.0 g), m.p. 114 - 115° (reported[32], m.p. 116°).

3β-Acetoxy-5, 6α-epoxy-5α-cholestane (XII –c) :

3β-Acetoxycholest-5-ene (LXXII) (10 g) in chloroform (100 ml) was treated with a solution of perbenzoic acid (1.1 mol equivalent) in chloroform and left for 20 hrs. at a temperature of -8°. After the completion of reaction, the reaction mixture was washed successively with ice-cold sodium bicarbonate solution (5%), water, sodium thiosulphate solution (5%) and water. Evaporation of the solvent gave an oil which was chromatographed over silica gel column. Elution with petroleum ether – ether (10:1) gave a solid compound which was recrystallized from acetone as needles to afford epoxide (XII-c) (8.0 g), m.p. 97° (reported[32], m.p. 97°).

Reaction of 5, 6α-epoxide-5α-cholestane (XII-a) in DMF with Acetamide (anhydrous $AlCl_3$ used as catalyst) : 5α-Cholestano [5, 6α-d]-2′-methyl-2-oxazoline (LXII-a) and 5, 6α-hydroxy-6β-cholestane (XIII-a) :

5,-6α-Epoxy-5α-cholestane (XII-a) (2.0 g) and acetamide (1.0 g) were dissolved in DMF (50 ml) was heated under reflux for 20 hrs. (anhydrous $AlCl_3$ was used as catalyst). The progress of reaction was monitored by TLC. After the completion of the reaction, the solvent was removed under reduced pressure and the residue thus obtained was extracted with ether. The ethereal layer was washed several times with water and dried over anhydrous sodium sulphate, Removal of the solvent gave semi solid (1.73 g) which was chromatographed over silica gel (40 g).

5α-Cholestano[5, 6α-d]-2′-methyl-2-oxazoline (LXII-a) :

Elution : pet. ether : ether (15:1), solvent of crystallization : Petroleum ether, Yield : (0.83 g), m.p. 101°.

Analysis Found : C, 81.47; H, 11.46; N, 3.26

$C_{29}H_{49}NO$ requires : C, 81.49; H, 11.47; N, 3.27%

IR : ν max 1680 (C=N), 1350 (C-N) and 1050 cm^{-1}

^{1}H-NMR($CDCl_3$) : δ 4.78 (1H, dd, Jaa = 11 Hz, Jee 4 Hz, H-6β), 2.13 (s, 3H, CH_3-C=N), 1.01 (C10-CH_3), 0.68 (C13-CH_3), 0.95 and 0.85 (side chain methyl protons)

Mass : m/z 427 (M^+), m/z 410, m/z 57.

5,6β-Dihydroxy-5α-cholestane (XIII-a) :

Elution : Pet.ether : ether (10:1), solvent of crystallization : methanol, Yield : (0.45 g), m.p. (reported[38] m.p. 125.5°)

Analysis Found : C, 80.17; H, 11.97

$C_{27}H_{48}O_2$ requires : C, 80.19; H, 11.99%

On the basis of m.p. m.m.p. TLC, IR, ^{1}H-NMR and mass spectral values which were found identical with authentic sample[38], the compound was characterized as 5, 6β-dihydroxy-5α-cholestane (XIII-a).

Reaction of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) with Acetamide-DMF (anhydrous $AlCL_3$ as catalyst) : 3β-Chloro-5α-

cholestano [5, 6α-d]-2′-methyl-2-oxazoline (LXII-b) and 3β-chloro-5, 6β-dihydroxy-5α-cholestane (XIII-b) :

3β-Chloro-5, 6α-epoxy-5α-cholestane (XII-b) (2.0 g) dissolved in DMF (50 ml) was treated with acetamide (1.0 g) anhydrous $AlCl_3$ as catalyst. After the completion of reaction, monitored by TLC, the solvent was removed and the residue (1.62) was work up and column chromatographed (35 g, silica gel).

3β-Chloro-5α-cholestano [5,6α-d]-2′-methyl-2-oxazoline (LXII-b):

Elution : pet.ether : ether (12:1), solvent of crystallization : petroleum ether, Yield : (0.84 g), m.p. 110°.

Analysis Found : C, 75.46; H, 10.40; N, 3.02

$C_{29}H_{48}NOCl$ requires : C, 75.48; H, 10.41; N, 3.02%

I.R : ν max. 1690 (C=N), 1360 (C-N), 1060 (C-O) and 710 cm^{-1} (C-Cl).

^{1}H-NMR($CDCl_3$) : δ 4.80 (1H; dd; Jaa 12; Jae = 4.5 Hz; H-6β), 3.90 (mc, 1H, W½ = 16 Hz, H-3α)[33] 2.15 (s, 3H, CH_3-C=N-), 1.02 (C10-CH_3), 0.65 (C13-CH_3), 0.98 and 0.88 (side chain methyl protons).

Mass : m/z 461/463 (M±), m/z 444/446, m/z 57.

3β-Chloro-5, 6β-dihydroxy-5α-cholestane (XIII-b) :

Elution : pet.ether : ether (8:1), solvent of crystallization : methanol. Yield : (0.55 g) m.p. 123° (reported[37] m.p. 125-126°).

Analysis Found : C, 73.95; H, 14.48

$C_{27}H_{47}O_2Cl$ requires : C, 73.97; H, 14.50%

On the basis of m.p., m.m.p., TLC, IR, ^{1}H-NMR and mass spectral values which found identical with authentic sample of chlorodiol the compound (XIII-b) was characterized as 3β-chloro-5, 6β-dihydroxy-5α-cholestane.

Reaction of 3β-acetoxy-5, 6α-epoxy-5α-Cholestane (XII-c) with acetamide in DMF (anhydrous $AlCl_3$ as catalyst) : 3β-Acetoxy-5α-cholestano [5, 6α-d]-2′-methyl-2-oxazoline (LXII-c) and 3β-acetoxy-5, 6β-dihydroxy-5α-cholestane (XIII-c).

3β-Acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) (2.0 g) and acetamide (1.0 g) were dissolved in DMF (50 ml) and heated under reflux (anhydrous

$AlCl_3$ was used as catalyst). The progress of the reaction was monitored by TLC. After the completion of the reaction, the solvent was removed under reduced pressure. The residue thus obtained was extracted with ether. The ethereal layer was washed several times with water and dried over anhydrous sodium sulphate. Removal of the solvent gave an oil (1.65 g) (LXII-c) which was chromatographed over silica gel (40 g).

3β-Acetoxy-5α-cholestano[5,6α-d]-2′-methyl-2-oxazoline (LXII-c):

Elution : pet.ether : ether (10:1) ,Yield : (0.78 g), semisolid.

Analysis Found : C, 76.69; H, 10.50; N, 2.86

$C_{31}H_{51}NO_3$ requires : C, 76.70; H, 10.51; N, 2.88%

IR ν max : 1735 (CH_3COO), 1685 (C=N), 1360 (C-N), 1270, 1030 cm^{-1} (C-O).

^{1}H-NMR ($CDCl_3$) : δ 4.40 (mc, 1H, W½ = 10 Hz, H-6β)[33], 4.10 (mc, 1H, 17 Hz, H-3α)[33], 2.30 (s, 3H, CH_3-C=N-), 2.10 (s, CH_3COO), 1.02 (C10-CH_3), 0.69 (C13-CH_3), 0.94 and 0.86 (side chain methyl protons).

Mass : m/z 473 (M^+), m/z 456 and m/z 57

3β-Acetoxy-5, 6β-dihydroxy-5α-cholestane (XIII-c) :

Elution : pet.ether : ether (8:1), solvent of crystallization : methanol, Yield : (0.63g), m.p 208° (reported[32] m.p.209).

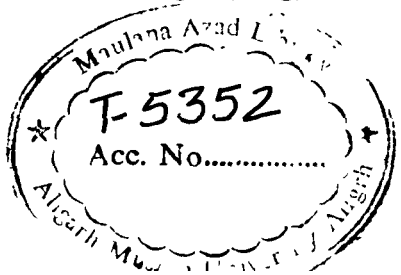
Analysis found : C, 75.30; H, 10.82%

$C_{29}H_{50}NO_4$ requires : C, 75.32; H, 10.82%

On the basis of m.p. m.m.p. TLC, IR, ^{1}H-NMR and mass spectral values which were found identical with the authentic sample, the compound is characterized as 3β-acetoxy-5,6β dihydroxy-5α-cholestane[3](XIII-C).

Reaction of 5, 6α-epoxy-5α-cholestane (XII-a) with benzamide in DMF (anhydrous $AlCl_3$ as catalyst) : 5α-cholestano [5,6α-d]–2′-phenyl–2-oxazoline (LXIII-a) and 5, 6β-dihydroxy-5α-cholestane (XIII-a) :

In 5, 6α-epoxy-5α-cholestane (XII-a) (2.0 g) dissolved in DMF (50 ml) was added in portions benzamide (1.0 g) (anhydrous $AlCl_3$ was used as catalyst) and heated under reflux for 25 hrs. After the completion of reaction monitored by TLC, the solvent was removed and the residue (1.80 g) was chromatographed over silica gel (40 g).



5α-Cholestano [5, 6α-d]-2′-phenyl-2-oxazoline (LXIII-a) :

Elution : pet.ether : ether (12:1), solvent of crystallization : petroleum ether, Yield : (0.80 g) m.p. 134°.

Analysis Found : C, 83.42; H, 10.40; N, 2.85

$C_{34}H_{51}NO$ requires : C, 83.43; H, 10.42; N, 2.86%

I .R ν max : 3100-300°(C-H, stretch; aromatic), 1680 (C=N), 1600-1590 (C=C), 1360 (C-N) and 1050 cm^{-1} (C-O).

^{1}H-NMR ($CDCl_3$) : δ 7.7 (br mc, 5-aromatic protons), 3.55 (dd, 1H, Jaa = 10 Hz, J = 3.5 Hz, H-6β)[33], 1.2 (C10-CH_3), 0.73 (C13-CH_3), 0.98, 0.85 (side chain methyl protons).

Mass : m/z 477 (M^+), m/z 460, m/z 119.

5, 6β-Dihydroxy-5α-cholestane (XIII-a) :

Elution : pet.ether : ether (10:1), solvent of crystallization : methanol, Yield : (0.43 g), m.p. 124° (reported[38] m.p. 125.5).

Analysis Found : C, 80.17; H, 11.97

$C_{27}H_{48}O_2$ requires : C, 80.19; H, 11.99%

On the basis of identical m.p., m.m.p., TLC, IR, ^{1}H-NMR and mass spectral values with authentic sample[38], the compound m.p. 124° was characterized as 5, 6β-dihydroxy-5α-cholestane (XIII-a).

Reaction of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) with benzamide in DMF ($AlCl_3$ as catalyst) : 3β-Chloro-5α-cholestano [5, 6α-d]-2′-phenyl-2-oxazoline (LXIII-b) and 3β-chloro-5, 6β-dihydroxy-5α-cholestane (XIII-b) :

3β-Chloro-5, 6α-epoxy-5α-cholestane (XII-b) (2.0 g) dissolved in DMF (50 ml) was treated with benzamide (1.0 g), anhydrous $AlCl_3$ was used as catalyst agent under reflux condition for 20 hrs. After the completion of reaction monitered by TLC, the solvent was removed and the residue (1.65 g) was work up and chromatographed over silica gel (36.0 g).

3β-Chloro-5α-cholestano[5,6α-d]-2′-phenyl-2-oxazoline (LXIII-b):

Elution : pet.ether : ether (10:1), solvent of crystallization : Petroleum ether, Yield : (0.80 g), m.p. 157°.

Analysis Found : C, 78.00; H, 9.55; N, 2.66

$C_{34}H_{50}NOCl$ requires : C, 78.01; H, 9.56; N, 2.67%

IR ν max : 3150-3070 (C-H, stretch, aromatic), 1685 (C=N), 1620 (C=C), 1365 (C-N), 1060 (C-O-C) and 715 cm^{-1} (C-Cl)

^{1}H-NMR ($CDCl_3$) : δ 7.78 (br. mc, 5H, aromatic protons), 3.90 (dd, Jaa = 11, Jae = 3.5 Hz (H-6β), 3.95 (mc, 1H, W½ = 18 Hz, H-3α)[33], 1.10 (C10-CH_3), 0.70 (C13-CH_3), 0.92, 0.87 (side chain methyl protons).

Mass : m/z 523/525 (M^+), m/z 506/508, m/z 119.

3β-Chloro-5, 6β-dihydroxy-5α-cholestane (XIII-b) :

Solvent of elution : Pet.ether : ether (8:1), solvent of crystallization : methanol, Yield : (0.57 g) m.p. 123° (reported[37] m.p. 125-126°).

Analysis Found : C, 73.94; H, 14.49

$C_{27}H_{47}O_2Cl$ requires : C, 73.95; H, 14.50%

On the basis of identical m.p., m.m.p., TLC, IR, ^{1}H-NMR, mass spectral values with authentic sample[37], the compound m.p. 123° was characterized as 3β-chloro-5, 6β-dihydroxy-5α-cholestane (XIII-b).

Reaction of 3β-acetoxy-5, 6β-epoxy-5α-cholestane (XII-c) with benzamide in DMF (anhydrous $AlCl_3$ used as catalyst) : 3β-Acetoxy-5α-cholestano [5, 6α-d]-2′-phenyl-2-oxazoline (LXIII-c) and 3β-acetoxy-5, 6β-dihydroxy-5α-cholestane (XIII-c) :

In 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) (2.0 g) dissolved in DMF (50 ml) was added benzamide (1.0 g) and small amount of anhydrous $AlCl_3$ as catalyst. The reaction mixture was heated under reflux for 20 hrs. The progress of reaction was monitored by TLC. After the completion of the reaction, the solvent was removed under reduced pressure and the residue thus obtained (1.70 g) was chromatographed over silica gel (40 g).

3β-Acetoxy-5α-cholestano [5, 6α-d]-2′-oxazoline (LXIII-c) :

Elution pet.ether : ether (10:1), solvent of crystallization : petroleum ether, Yield : (0.82 g) m.p. 220°.

Analysis Found : C, 78.96; H, 9.67 N, 2.54

$C_{36}H_{53}NO_3$ requires : C,78.97; H, 9.68; N, 2.55%.

IR : ν max 3150-3060 (C-H, stretch, aromatic), 1740 (CH_3COO), 1665 (C=N), 1615-1585 (C=C), 1365 (C-N), 1260, 1025 cm^{-1} (C-O).

^{1}H-NMR ($CDCl_3$) : δ 7.9 (br mc, 5-aromatic protons), 4.35 (mc, 1H, W½ = 12 Hz, H-6β)[33], 4.25 (mc, 1H, 18 Hz, H-3α)[33], 2.15 (s, CH_3COO), 1.12 (C10-CH_3), 0.72 (C13-CH_3), 0.96 and 0.85 (side chain methyl protons).

Mass : m/z 547 (M^+), m/z 530, m/z 119.

3β-Acetoxy-5, 6β-dihydroxy-5α-cholestane (XIII-c) :

Elution : pet.ether : ether (8:1), solvent of crystallization : methanol, Yield : (0.65 g) m.p. 208° (reported[32] m.p. 209°).

Analysis Found : C, 78.96; H, 9.97

$C_{36}H_{53}NO_3$ requires : C, 78.97; H, 9.68%

On the basis of m.p., m.m.p., TLC, IR, ^{1}H-NMR and mass spectral values, the compound was characterized as 3β-acetoxy-5, 6β-dihydroxy-5α-cholestane (XIII-c).

Reaction of 5, 6α-epoxy-5α-cholestane (I) with semicarbazide : 5-Hydroxy-6β-amino-5α-cholestane (LXIV-a) : 5-hydroxy-6β-amino-N-formyl-cholestane (LXIV-b) and N-amido-5α-cholestano [5, 6-b]-aziridine (LXIV-c).

The mixture of 5, 6α-epoxy-5α-cholestane (XII-a) (1.5 g) and semicarbazide (1.5 gm) was dissolved in dimethyl formamide (30 ml) anhydrous $AlCl_3$ as catalyst) and it was heated under reflux for 8 hrs. The reaction was monitored with TLC till all the starting material was consumed. After the completion of the reaction, the reaction mixture was poured in water and extracted with ether. The ethereal layer was washed with water several times to remove unreacted semicarbazide and dried over anhydrous sodium sulphate. The solvent was evaporated on water bath to yield a residue in the form of an oily mass (1.3 gm) which was chromatographed over silic gel (25 gm).

5-Hydroxy-6β-amino-5α-cholestane (LXIV-a) :

Elution : pet.ether : ether (15:1), solvent of crystallization : petroleum ether, yield : (0.53 g), m.p. 137°.

Analysis Found : C, 80.37; H, 12.16; N, 3.45%

$C_{27}H_{49}NO$ requires : C, 80.39; H, 12.15; N, 3.47%

IR : ν max 3480 (-NH, -OH), 1280 and 1040 (C-O).

^{1}H-NMR ($CDCl_3$) : δ4.5 (brs, 2H, $-NH_2$), 4.0 (brs, -OH, exchangeable with D_2O), 3.75 (mc, H-6α), 1.02 (C10-CH_3), 0.65 (C13 – CH_3), 0.85 and 0.77 (side chain methyl protons).

MS : m/z 403 (M^+), m/z 388 (M^+ - CH_3), m/z 385 (M^+ - H_2O) and m/z 290 (M^+ - C_8H_{17}).

5-Hydroxy-6β-amino-N-formyl-5α-cholestane (LXIV-b) :

Elution : Pet.ether : ether (10:1), solvent of crystallization : Petroleum ether, Yield : (0.3g), m.p. 121°.

Analysis Found : C, 77.98; H, 11.35; N, 3.25

$C_{28}H_{49}NO_2$ requires : C, 77.95; H, 11.36; N, 3.28%.

IR : ν max 3510, 3485 (-NH, -OH), 1690 (H-C=O), 1390cm^{-1} (C-O).

^{1}H-NMR ($CDCl_3$) : δ 8.5 (brs, 1H, H-C=O), 5.75 (brs, 1H, -N-H, exchangeable with deuterium), 3.5 (mc, 1H, H-6α, equatorial), 2.30 (brs, 1H, exchangeable with deuterium -OH), 1.1 (C10-CH_3), 0.73 (C13-CH_3), 0.92 and 0.83 (side chain methyl protons).

Mass : m/z 431 (M+), m/z 413 ($M^{+}_{\bullet}$ -H_2O), m/z 403 (M+ -CO) and lower mass peaks.

N-Amido-5α-cholestano [5, 6-b]-aziridine (LXIV - c)

Elution : Pet. ether : ether (5:1), solvent of crystallization : methanol, Yield : (0.27 g), m.p. 115°.

Analysis Found : C, 78.48; H, 11.20; N, 6.53

$C_{28}H_{48}N_2O$ requires : C, 78.50; H, 11.21; N, 6.54%

IR : ν max 3450 (-NH), 1685(-NH-CO), and 1390 cm^{-1} (C-N).

^{1}H-NMR ($CDCl_3$) : δ 5.1 (brs, 2H, -NH_2), 3.1 (mc, 1H, H-6β), 1.01 (C10-CH_3), 0.65 (C13-CH_3), 0.92 and 0.85 (side chain methyl protons)

MS : m/z 428 ($M^{+}_{\bullet}$), m/z 427 ($M^{+}_{\bullet}$ -H), m/z 413 ($M^{+}_{\bullet}$ -CH_3) and m/z 315 ($M^{+}_{\bullet}$ -C_8H_{17}).

Reaction of 5, 6α-epoxy-5α-cholestane (XII-a) with phenyl semicarbazide : 5-Hydroxy-6β-amino-5α-cholestane (LXIV-a), 5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXIV-b) and N-amido-5α-cholestano [5, 6-b]-aziridine (LXIV-c).

The mixture of 5, 6α-epoxy-5α-cholestane (I) (2.0 gm) and phenyl semicarbazide (2.0 gm) was dissolved in dimethyl formamide (40 ml) (anhydrous $AlCl_3$ as catalyst) and it was heated under reflux for 8 hrs. The reaction was monitored with TLC till all the starting material was consumed. After the completion of the reaction, the reaction mixture was poured into water and extracted with ether. The ethereal layer was washed with water several times to remove unreacted phenyl semicarbazide and dried over anhydrous sodium sulphate. The solvent was evaporated on a water bath to yield a residue in the form of an oily mass (1.5 g) which was chromatographed over silica gel (30 g).

5-Hydroxy-6β-amino-5α-cholestane (LXIV-a) :

Elution : Pet.ether : ether (15:1), solvent of crystallization : Petroleum ether, Yield : (0.45 g), m.p. 137°.

Analysis Found : C, 80.36; H, 12.14; N, 3.46

$C_{27}H_{49}NO$ requires : C, 80.39; H, 12.15; N, 3.47%.

The m.p., m.m.p., TLC, IR, ^{1}H-NMR and Mass spectral data were found identical with the hydroxyamino compound (LXIV-a) which was obtained when 5, 6α-epoxy-5α-cholestane (XII-a) was treated with semicarbazide under identical reaction conditions.

5-Hydroxy-6β-amino-N-formyl-5α-cholestane (LXIV-b) :

Elution : Pet.ether : ether (10:1), solvent of crystallization : Petroleum ether, Yield : (0.35 g), m.p. 121°.

Analysis Found : C, 77.94; H, 11.37; N, 3.26.

$C_{28}H_{49}NO_2$ requires : C, 77.95; H, 11.36; N, 3.28%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data of amino formyl compound (LXIV-b) were found identical with the same compound obtained when epoxide (XII-a) was treated with semicarbazide under similar reaction conditions.

N-Amido-5α-cholestano [5, 6-d]-aziridine (LXIV-c) :

Elution : Pet.ether : ether (5:1), solvent of crystallization : Petroleum ether, Yield : (0.5 g), m.p. 115°.

Analysis Found : C, 78.47; H, 11.19; N, 6.52.

$C_{27}H_{49}N_2O$ requires : C, 78.50; H, 11.21; N, 6.54%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data of amino formyl compound (LXIV-c) were found identical with the same compound obtained when epoxide (XII-a) was treated with semicarbazide under similar reaction conditions.

Reaction of 5, 6α-epoxy-5α-cholestane (XII-a) with semithiocarbazide : 5-Hydroxy-6β-amino-5α-cholestane (LXIV-a), 5-hydroxy-6β-amino-N-thioformyl-5α-cholestane (LXV-a) and N-thioamido-5α-cholestano [5, 6-b]-aziridine (LXV-b).

The mixture of 5, 6α-epoxy-5α-cholestane (XII-a) (2.2 gm) and semithiocarbazide (2.2 gm) was dissolved in dimethyl formamide (40 ml) (anhydrous $ALCl_3$ was used as catalyst) and it was heated under reflux for 8 hrs. The reaction was monitored with TLC. After the completion of the reaction, the reaction mixture was poured in water and extracted with ether. The ethereal layer was washed with water several times to remove unreacted semithiocarbazide and dried over anhydrous sodium sulphate. The solvent was evaporated on a water bath to yield a residue in the form of an oily mass (1.8 gm) which was chromatographed over silica gel (36 gm).

5-Hydroxy-6β-amino-5α-cholestane (LXIV-a) :

Elution : Pet.ether : ether (15:1), solvent of crystallization : Petroleum ether, Yield : (0.40 g), m.p. 138°.

Analysis Found : C, 80.38; H, 12.13; N, 3.45.

$C_{27}H_{49}NO$ requires : C, 80.39; H, 12.15; N, 3.47%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data were found identical with amino compound (LXIV-a) which was obtained when epoxide (XII-a) was treated with semicarbazide or phenyl semicarbazide under same reaction conditions.

5-Hydroxy-6β-amino-N-thioformyl-5α-cholestane (LXV-a) :

Elution : Pet.ether : ether (10:1), Yield :(0.45 g), m.p. 198°.

Analysis Found : C, 75.05; H, 11.01; N, 3.10

$C_{28}H_{49}NSO$ requires : C, 75.10; H, 11.03; N, 3.12%.

IR : ν max 3545 – 3450 (-OH, -NH), 1520(-C=S)[34] and 1360 cm^{-1} (C-N).

^{1}H-NMR ($CDCl_3$) : δ 7.4 (H, H-C=S), 5.76 (brs, 1H, exchangeable with deuterium, -N-H), 3.85 (mc, 1H, W½ = 6 Hz, H-6α, equatorial), 2.65 (brs, exchangeable with deuterium, -OH), 1.13 (C10-CH_3), 0.65 (C13, CH_3), 0.96 and 0.86 (side chain methyl protons).

Mass : m/z 447 (M^+), m/z 432 (M^+ - CH_3), m/z 429 (M^+ - H_2O), m/z 403 (M^+ -C=S) and lower fragment ion mass peaks.

N-Thioamido-5α-cholestano [5, 6-b]-aziridine (LXV-b) :

Elution : Pet.ether : ether (5:1), solvent of crystallization : methanol, Yield : (0.70 g), m.p. 124°.

Analysis Found : C, 75.64; H, 10.0; N, 6.28; S, 7.18.

$C_{28}H_{48}N_2S$ requires : C, 75.67; H, 10.01; N, 6.30; S, 7.20%

IR : ν max 3425 (-N-H),1525 (C=S), 1360 cm^{-1} (C-N.

^{1}H-NMR ($CDCl_3$) : δ5.9 (s, 2H, $-NH_2$), 3.8 (mc, 1H, W½ = 10.5 Hz, H-6), 1.01 (C10 – CH_3), 0.65 (s, C13 – CH_3), 0.92 and 0.86 (Side chain methyl protons).

Mass : m/z 444 ($M^{+\bullet}$), m/z 429 ($M^{+\bullet}$ - CH_3), m/z 427 ($M^{+\bullet}$ - NH_3), m/z 400 ($M^{+\bullet}$ - C=S) and lower fragment ion mass peaks.

Reaction of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) with semithiocarbazide : 3β-Chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a), 3β-chloro-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVI-b) and 3β-chloro-N-thio-amido-5α-cholestano[5, 6-b]-aziridine (LXVI-c).

The mixture of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) (1.5 g) and semithiocarbazide (1.5 g) dissolved in DMF (30 ml) (anhydrous $AlCl_3$ was used as catalyst) and it was heated under reflux for 8 hrs. the reaction was monitored by TLC and after completion of the reaction, the mixture was poured in water , worker up in usual, manner and dried over anhydrous sodium sulphate. The solvent was evaporated over water bath to yield a residue (1.4 gm) which was column chromatographed over silica gel (28 gm).

3β-Chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a) :

Elution : Pet.ether : ether (15:1), solvent of crystallization : Petroleum ether,

Yield : (0.35 g), m.p. 173° (positive Beilstein test)

Analysis Found : C, 74.16; H, 10.97; N, 3.21%

$C_{27}H_{48}NOCl$ requires : C, 74.14; H, 10.98; N, 3.20%.

IR : ν max 3585 – 3450 (-OH, -NH), 1395 (C-N) 760 cm^{-1} (C-Cl).

^{1}H-NMR ($CDCl_3$) : δ4.7 (brs, 2H, $-NH_2$), 4.1 (mc, 1H, W½ = 17 Hz, axial, H - 3α), 3.6 (mc, 1H, W½ = 5.5 Hz, equatorial, H - 6α), 0.93 and 0.87 (side chain methyl protons).

Mass : m/z 437/439 ($M^{+\bullet}$), m/z 422/424 ($M^{+\bullet}$ -CH_3), m/z 419/421 ($M^{+\bullet}$ -H_2O), m/z 383 (m/z 419 - HCl), m/z 366 (m/z 383 – NH_3) and fragment ions of lower mass.

3β-Chloro-5-hydroxy-6β-amino-N-formyl-5α-cholestane(LXVI-b):

Elution : Pet.ether : ether (9:1), solvent of crystallization : Petroleum ether, Yield : (0.43 g), m.p. 153° (positive Beilstein test).

Analysis Found : C, 72.23; H, 10.30; N, 3.00

$C_{28}H_{48}NO_2Cl$ requires : C, 72.25; H, 10.32; N, 3.01 %.

IR : ν max 3590 – 3445 (-OH, -NH), 1695 (-C=O), 1405 (C-N) and 730 cm^{-1} (C-Cl).

^{1}H-NMR ($CDCl_3$) : δ 8.2 (brs, 1H, H-C=O), 5.6 (mc, 1H, exchangeable with deuterium, -NH), 3.48 (mc, 2H, H-3α and H-6α), 1.2 (C10 – CH_3), 0.7 (C13 – CH_3), 0.93 and 0.83 (side chain methyl protons).

Mass : m/z 465/467 ($M^{+\bullet}$), m/z 450/452 ($M^{+\bullet}$ - CH_3), m/z 447/449 ($M^{+\bullet}$ - H_2O), m/z 429 ($M^{+\bullet}$ - HCL) and fragment ions of lower mass.

3β-Chloro-N-thioamido-5α-cholestano[5,6-b]-aziridine (LXVI-c):

Elution : Pet.ether : ether (5:1), solvent of crystallization : methanol, Yield : (0.45 g) , m.p. 132° (positive Beilstein test).

Analysis Found : C, 72.70; H, 10.22; N, 6.04

$C_{28}H_{47}N_2OCl$ requires : C, 72.72; H, 10.24; N, 6.06%.

IR : ν max 3460 (-NH),1690 (-NH-CO), 1395 (C-N), 765 cm^{-1} (C-Cl).

^{1}H-NMR ($CDCl_3$) : δ 5.3 (s, 2H, exchangeable with deuterium, $-HN_2$) 4.3 (mc, 1H, W½ = 18 Hz, H-3α), 3.90 (mc, 1H, W½ = 6.2 Hz, H-6β, axial), 1.1 (C10 – CH_3), 0.70 (C13 – CH_3), 0.95 and 0.88 (side chain methyl protons).

Mass : m/z 462/464 ($M^{+\cdot}$), m/z 447/449 ($M^{+\cdot}$ -CH_3), m/z 445/447 ($M^{+\cdot}$ - NH_3), m/z 426 ($M^{+\cdot}$ - HCl), m/z 418/420 ($M^{+\cdot}$ - NH_2CO) and fragment ions of lower mass.

Reaction of 3β-chloro-5,6-epoxy-5α-cholestane (XII-b) with phenyl semicarbazide : 3β-Chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a), 3β-chloro-5-hydroxy-amino-N-formyl-5-cholestane (LXVI-b) and 3β-chloro-N-amido-5α-cholestane [5,6-b]-aziridine (LXVI-c).

The mixture of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) (2.0 g) and phenyl semicarbazide (2.0 g) was dissolved in dimethyl formamide (35 ml) (anhydrous $AlCl_3$ as catalyst) and heated under reflux for 8 hrs. The reaction mixture was monitored by TLC and after completion of reaction, the reaction mixture was poured in water, worked up in usual manner and dried over anhydrous sodium sulphate. The solvent evaporation over water bath yielded a residue (1.58 g) which was column chromatographed over silica get (32 g).

3β-Chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a) :

Elution : Pet.ether : ether (15:1), solvent of crystallization : Petroleum ether, Yield :(0.37 g), m.p. 173° (positive Beilstein test).

Analysi Found : C, 74.12; H, 10.96; N, 3.19.

$C_{27}H_{48}NOCL$ requires : C, 74.14; H, 10.98; N, 3.20%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data were found identical with hydroxy amino compound (LXVI-a), found when 3β-chloro-epoxy-5α-cholestane (XII-b) was treated with semicarbazide under similar reaction conditions.

3β-Chloro-5-hydroxy-6β-amino-N-formyl-5α-cholestane(LXVI-b):

Elution : Pet.ether : ether (10:1), solvent of crystallization : Petroleum ether, Yield : (0.50 g), m.p. 153° (positive Beilstein test).

Analysis Found : C, 72.24; H, 10.31; N, 3.0

$C_{27}H_{48}NO_2CL$ requires : C, 72.2; H, 10.32; N, 3.01%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data were found identical with hydroxy amino compound (LXVI-b), obtained when 3β-chloro-5, 6β-epoxy-5α-cholestane (XII-b) was treated with semicarbazide under similar reaction conditions.

3β-Chloro-N-amido-5α-cholestano [5, 6-b]-aziridine (LXVI-c) :

Elution : Pet.ether : ether (5:1), solvent of crystallization : methanol, Yield : (0.5 g), m.p. 132° (positive Beilstein test).

Analysis Found : C, 72. 69; H, 10.23; N, 6.05.

$C_{28}H_{47}N_2OCL$ requires : C, 72.72; H, 10.24; N, 6.06%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data were found identical with the amino aziridine obtained when epoxide (XII-b) was treated with semicarbazide under similar reaction conditions.

Reaction of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-b) with semithiocarbazide : 3β-Chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a), 3β-chloro-5-hydroxy-6β-amino-N-thioformyl-5α-cholestane (LXVII-a) and 3β-Chloro-N-thioamido-5α-cholestano [5, 6-b]-aziridine (LXVII-b).

The mixture of 3β-chloro-5, 6α-epoxy-5α-cholestane (XII-a) (2.5 g) and semithiocarbazide (2.5 g) was dissolved in dimethlformamide (40 ml) and was heated (anhydrous $AlCl_3$ as catalyst) under reflux for 8 hrs. The reaction was monitored by TLC and after completion of the reaction, the reaction mixture was poured in water, worked up in usual manner and dried over anhydrous sodium sulphate. The solvent evaporation over water bath yielded a residue (1.7 gm) which was column chromatographed over silica gel (35 gm).

3β-Chloro-5-hydroxy-6β-amino-5α-cholestane (LXVI-a) :

Elution : Pet.ether : ether (15:1), solvent of crystallization : Petroleum ether, Yield : (0.47 g), m.p. 172 - 173° (positive Beilstein test).

Analysis Found : C, 74.13; H, 10.97; N, 3.18

$C_{27}H_{48}NOCL$ requires : C, 74.14; H, 10.98; N, 3.20%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data were found identical with hydroxy amino compound (LXVI-a), found when epoxide (XII-b) was treated with semicarbazide under similar reaction conditions.

3β-Chloro-5-hydroxy-6β-amino-N-thioformyl-5α-cholestane (LXVII-a) :

Elution : Pet.ether : ether (10:1), solvent of crystallization : Petroleum ether, Yield : (0.35 g), oil.

Analysis Found : C, 69.74; H, 10.01; N, 2.88; S, 6.0

$C_{28}H_{48}NOSCl$ requires : C, 69.74; H, 10.03; N, 2.90; S, 6.26%.

IR : ν max 3560 - 3435 (-OH, -NH), 1530 (H-C=S), 1390 (C-N), 740 cm^{-1} (C-Cl).

^{1}H-NMR ($CDCl_3$) : δ 8.1 (s, 1H, H-C=S), 5.35 (brs, 1H, exchangeable with deuterium), 4.30 (mc, 1H, W½ = 16 Hz, H-3α), 3.75 (mc, 1H, W½ = 6 Hz, H - 6α), 2.25 (brs, 1H, -OH), 1.15 (C10 – CH_3), 0.70 (C13 – CH_3), 0.95 and 0.85 (side chain methyl protons).

Mass : m/z 481/483 ($M^{+\cdot}$), m/z 466/468 ($M^{+\cdot}$ - CH_3) m/z 463/465 ($M^{+\cdot}$ - H_2O), m/z 455 ($M^{+\cdot}$ - HCl) and fragment ion peaks of lower mass.

3β-Chloro-N-thioamido-5α-cholestano[5,6-b]-aziridine(LXVII-b) :

Elution : Pet.ether : ether (5:1), solvent of crystallization : methanol, Yield : (0.55 g), m.p. 141 - 142° (positive Beilstein test).

Analysis Found : C, 70.27; H, 9.81; 5.83; S, 6.68

$C_{28}H_{47}N_2SCL$ requires : C, 70.29; H, 9.83; N, 5.85; S, 6.69%.

IR : ν max 3435 (-NH), 1530 (C=S), 1310 (C-N), 765 cm^{-1} (C-Cl).

^{1}H-NMR ($CDCl_3$) : δ 5.4 (s, 2H, $-HN_2C=S$) 3.38 (mc, 1H, W½ = 6.5 Hz, H-6β), 3.55 (mc, 1H, W½ = 17 Hz, H-3α), 1.15 (C10-CH_3), 0.73 (C13 – CH_3), 0.93 and 0.88 (side chain methyl protons).

Mass : m/z 478/480 (M^+), m/z 463/465 ($M^+ - CH_3$), m/z 461/463 ($M^+ - NH_3$), m/z 434/436 ($M^+ - NH_2CO$), m/z 442 ($M^+ - HCl$) and lower mass peaks.

Reaction of 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) with semicarbazide : 3β-Acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a), 3β-acetoxy-5-hydroxy-6β-amino-N-formyl-5α-cho-

lestane (LXVIII-b) and 3β-acetoxy-N-amido-5α-cholestano [5, 6-b]-aziridine (LXVIII-c).

The mixture of 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) (1.5 g) and semicarbazide (1.5 g) was dissolved in dimethylformamide (25 ml) (anhydrous $AlCl_3$ as catalyst) and was refluxed for 8 hrs. The progress of the reaction was monitored with TLC, when all of the starting material was consumed, the reaction mixture was worked up in ether and dried over anhydrous sodium sulphate. On the solvent evaporation, the residue (1.30 gm) left was column chromotographed over silica gel (30 g).

3β-Acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a) :

Elution : Pet.ether : ether (12:1), solvent of crystallization : Petroleum ether, Yield : (0.38 g), m.p. 225 - 227°.

Analysis Found : C, 75.46; H, 11.05; N, 3.02 %.

$C_{29}H_{51}NO_3$ requires : C, 75.48; H, 11.06; N. 3.02 %.

IR : ν max 3530 - 3485 (-OH, -NH), 1730 ($-COCH_3$), 1365 (C-N), 1285 and 1045 cm^{-1} (C-O).

^{1}H-NMR ($CDCl_3$) : δ5.15 (mc, 1H, W½ = 18 Hz, H-3α), 2.75 (brs, 2H, $-NH_2$), 3.1 (mc, 1H, W½ = 6.8 Hz, H-6α), 2.75 (brs, 1H, -OH), 2.08 (s,

3H, -O-CO-CH_3), 1.15 (C10 – CH_3), 0.68 (C13 – CH_3), 0.97 and 0.85 (side chain methyl protons).

Mass : m/z 461 ($M^{+\cdot}$), m/z 446 ($M^{+\cdot}$ - CH_3), m/z 444 ($M^{+\cdot}$ - NH_3), m/z 443 ($M^{+\cdot}$ -H_2O).

3β-Acetoxy-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVIII-b) :

Elution : Pet.ether : ether (8:1), solvent of crystallization : Petroleum ether, Yield : (0.32 g), m.p. 200 - 203°.

Analysis Found : C, 73.60; H, 10.41; N, 2.84

$C_{30}H_{51}NO_4$ requires : C, 73.61; H, 10.42; N, 2.86%

IR : ν max 3510 - 3450 (-OH, -NH), 1725 (-$COCH_3$), 1330 (C-N), 1280, 1050 cm^{-1} (C-O).

^{1}H-NMR ($CDCl_3$) : δ 8.2 (s, 1H, H-C=O), 5.5 (mc, 1H, exchangeable with deuterium, -NH), 5.1 (mc, 1H, W½ = 18 Hz, H-3α), 3.8 (mc, 1H, W½ = 6 Hz, H-6α), 2.1 (s, 3H, -O-$COCH_3$), 1.13 (C10 – CH_3), 0.72 (C13 – CH_3), 0.91 and 0.88 (side chain methyl protons).

Mass : m/z 489 ($M^{+\cdot}$), m/z 474 ($M^{+\cdot}$ -CH_3), m/z 471 ($M^{+\cdot}$ -H_2O), m/z 429 ($M^{+\cdot}$ -CH_3COOH) and fragment ion peaks of lower mass.

3β-Acetoxy-N-amino-5α-cholestano[5,6-b]-aziridine(LXVIII-c):

Elution : Pet.ether : ether (4:1), solvent of crystallization : methanol, Yield : (0.45 g), m.p. 177 - 178°.

Analysis Found : C, 74.07; H, 10.28; N, 5.76%.

$C_{30}H_{50}N_2O_3$ requires : C, 74.07; H, 10.28; N, 5.76%

IR : ν max 3470 (-NH), 1735(-O-CO-CH_3), 1695 (-NH-CO), 1390 (C-N) 1240, 1040 cm^{-1} (C-O).

^{1}H-NMR ($CDCl_3$) : δ 5.20 (s, 2H, -NH_2), 5.05 (mc, 1H, W½ = 18 Hz, H-3α, axial), 4.05 (mc, 1H, W½ = 11Hz, H-6β), 2.30 (s, 3H, -O-CO-CH_3), 1.1 (C10 – CH_3), 0.74 (C13 – CH_3), 0.97 and 0.85 (side chain methyl protons).

Mass : m/z 486 (M^+), m/z 471 (M^+ -CH_3), m/z 442 (M^+ -NH_2CO), m/z (M^+ -CH_3COOH) and fragment ion peaks of lower mass.

Reaction of 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) with phenyl semicarbazide : 3β-Acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a), 3β-acetoxy-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVIII-b) and 3β-acetoxy-N-amido-5α-cholestano [5, 6-b]-aziridine (LXVIII-c).

The mixture of 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) (2.0 g) and phenyl semicarbazide (2.0 g) was dissolved in dimethyl formamide (30 ml) anhydrous $AlCl_3$ as catalyst and was refluxed for 8 hrs. The progress of the reaction was monitored with TLC, when all of the starting material was consumed, the reaction mixture was worked up in ether and dried over anhydrous sodium sulphate. On the solvent evaporation, the residue (1.5 gm) left was column chromatographed over silica gel (30 gm).

3β-Acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a) :

Elution : Pet.ether : ether (12:1), solvent of crystallization : Petroleum ether, Yield : (0.35 g), m.p. 225 - 227°.

Analysis Found : C, 75.47; H, 11.04; N, 3.01

$C_{29}H_{51}NO_3$ requires : C, 75.48; H, 11.06; N, 3.03%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data were found identical with the hydroxy amino compound (LXVIII-a), obtained when epoxide (XII-c) was treated with semicarbazide under same reaction conditions.

3β-Acetoxy-5-hydroxy-6β-amino-N-formyl-5α-cholestane (LXVIII-b) :

Elution : Pet.ether : ether (8:1), solvent of crystallization : Petroleum ether, Yield : (0.45 g), m.p. 200 - 203°.

Analysis Found : C, 73.59; H, 10.40; N, 2.85

$C_{30}H_{51}NO_4$ requires : C, 73.61; H, 10.42; N, 2.86%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data were found identical with the amino compound (LXVIII-b), obtained when epoxide (XII-c) was treated with semicarbazide under similar reaction conditions.

3β-Acetoxy-N-amino-5α-cholestano-[5, 6-b]-aziridine (LXVIII-c) :

Elution : Pet.ether : ether (4:1), solvent of crystallization : Petroleum ether, Yield : (0.45 g), m.p. 177 - 178°.

Analysis Found : C, 74.06; H, 10.27; N, 5.74

$C_{30}H_{50}N_2O_3$ requires : C, 74.07; H, 10.28; N, 5.76%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data were found identical with the amido azidirine (LXVIII-c), obtained when epoxide (XII-C) was treated with semicarbazide under similar reaction conditions.

Reaction of 3β-acetoxy-5, 6β-epoxy-5α-cholestane (XVI) with semithiocarbazides : 3β-Acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a), 3β-acetoxy-5-hydroxy-6β-amino-N-thioformyl-5α-cholestane (LXIX-a) and 3β-acetoxy-N-thioamido-5α-cholestano [5, 6-b]-aziridine (LXIX-b).

The mixture of 3β-acetoxy-5, 6α-epoxy-5α-cholestane (XII-c) (3.0 g) and phenyl semicarbazide (3.0 g) was dissolved in DMF (40 ml) (anhydrous $AlCl_3$ as catalyst) and was refluxed for 8 hrs. The progress of the reaction was monitored with TLC, when all of the starting material was consumed, the reaction mixture was worked up in ether and dried over anhydrous sodium sulphate. On the solvent evaporation, the residue (1.7 gm) left was column chromatographed over silica gel (35 gm).

3β-Acetoxy-5-hydroxy-6β-amino-5α-cholestane (LXVIII-a) :

Elution : Pet.ether : ether (15:1), solvent of crystallization : Petroleum ether, Yield : (0.38 g), m.p. 225 - 226°.

Analysis Found : C, 75.46; H, 11.04; N, 3.01

$C_{29}H_{51}NO_3$ requires : C, 75.48; H, 11.06; N, 3.03%.

The m.p., m.m.p., TLC, IR ^{1}H-NMR and Mass spectral data were found identical with the hydroxy amino compound (LXVIII-a), obtained when epoxide (XII-c) was treated with semicarbazide or semicarbazide under same reaction conditions.

3β-Acetoxy-5-hydroxy-6β-amino-N-thioformyl-5α-cholestane (LXIX-b) :

Elution : Pet.ether : ether (10:1), solvent of crystallization : Petroleum ether,

Yield : (0.42 g), m.p. 230°.

Analysis Found : C, 71.10; H, 10.14; N, 2.6; S, 6.32

$C_{30}H_{51}NSO_3$ requires : C, 71.12; H, 10.16; N, 2.7,; S, 6.33%.

IR : ν max 3510 - 3485 (-OH, -NH), 1735 (-O-CO-CH_3), 1525 (-C=S), 1370 (C-N), 1250, 1030 cm^{-1} (C-O).

^{1}H-NMR ($CDCl_3$) : δ 8.2 (s, 1H, H-C=S), 5.24 (brs, 1H, exchangeable with deuterium, -NH), 5.1 (mc, 1H W½ = 18 Hz, H-3α), 3.26 (mc, 1H, W½ = 6 Hz, H-6α), 2.27 (brs, 1H, -OH), 2.01 (s, 3H, -O-CO-CH_3), 1.15 (C10 – CH_3), , 0.68 (C13 – CH_3), 0.93 and 0.87 (side chain methyl protons).

Mass : m/z 505 (M^+), m/z 490 (M^+ -CH_3), m/z (M^+ -H_2O), m/z 445 (M^+ -CH_3COOH) and fragment ion peaks of lower mass.

3β-Acetoxy-N-thioamido-5α-cholestano-[5,6-b]-aziridine(LXIX-b):

Elution : Pet.ether : ether (5:1), solvent of crystallization : methonal, Yield : (0.50 g), m.p. 163 - 164°C.

Analysis Found : C, 71.70; H, 9.95; N, 5.56, S, 6.36

$C_{30}H_{50}SN_2O_2$ requires : C, 71.71; H, 9.96; N, 5.56; S, 6.37%.

IR : ν max 3440 (-NH), 1735 (-O-CO-CH_3), 1525 (-C=S), 1380 (C-N), 1240, 1025 cm^{-1} (C-O).

^{1}H-NMR ($CDCl_3$) : δ 5.4 (s, 2H, -NH_2-C=S), 5.1 (mc, 1H, W½ = 17.5 Hz, H - 3α), 3.16 (mc, 1H, W½ = 11 Hz, H-6β), 2.2 (s, 3H, -O-CO-CH_3), 1.2 (C10 – CH_3), 0.73 (C13 – CH_3), 0.96 and 0.85 (side chain methyl protons).

Mass : m/z 502 (M^+), m/z 487 (M^+ -CH_3), m/z 385 (M^+ -NH_3), m/z 458 (M^+ -C=S) and fragment ion peaks of lower mass.

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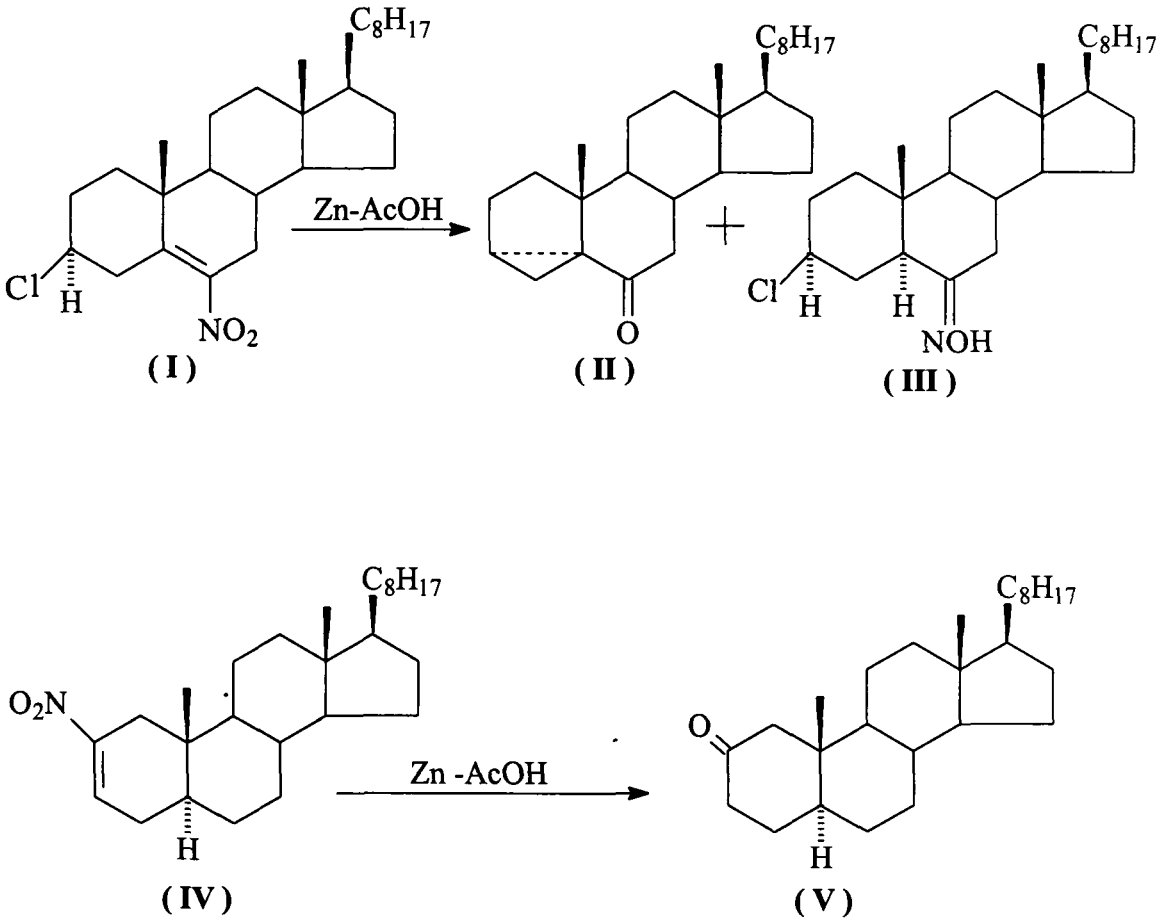
CHAPTER - 2

Reduction of Vinyl Nitro Steroids

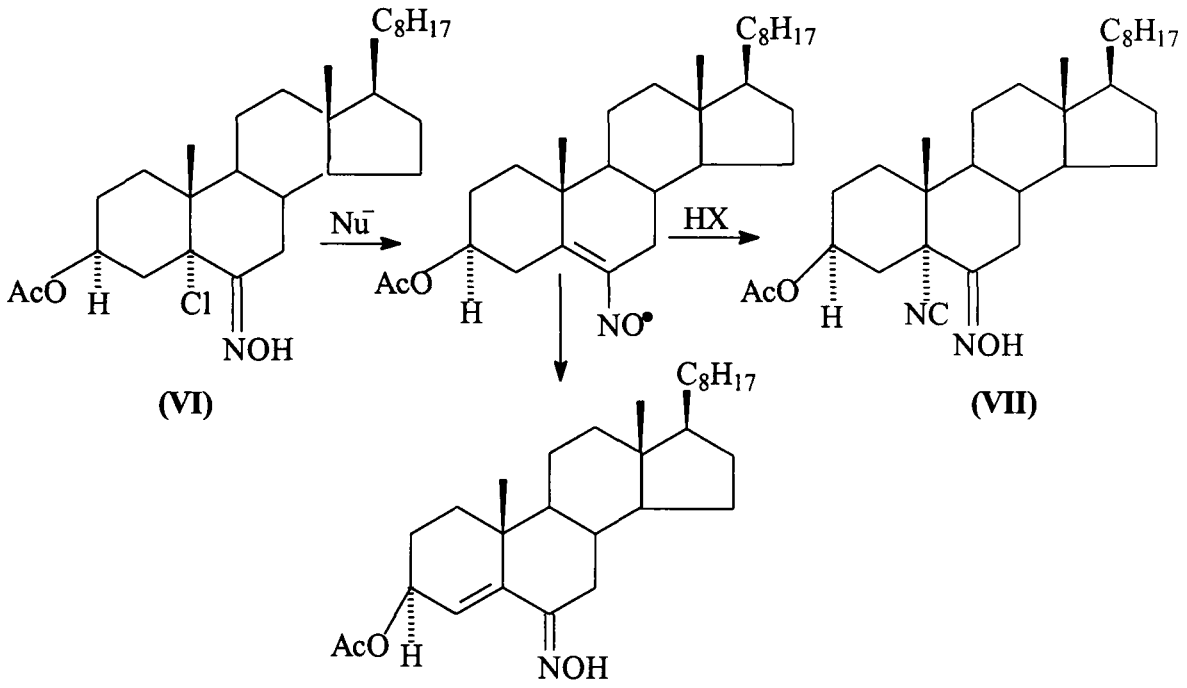
THEORETICAL

In the recent years synthesis of steroidal compounds have gained importance because of their biological activities associated with them. Reduction is one among the various reactions used in the synthetic pathway. Many types of reagents which have been successfully employed for this purpose are hydrogen with metal, lithium aluminium hydride, zinc-acetic acid, sodium borohydride and other metallic hydrides. Photochemical and electrochemical methods were also employed for reduction. Raney nickel catalysed reduction has not been studied thoroughly. To be very concise, in this chapter, attempts have been made to review[1] the various reagents used in the reduction of steroidal nitro compounds.

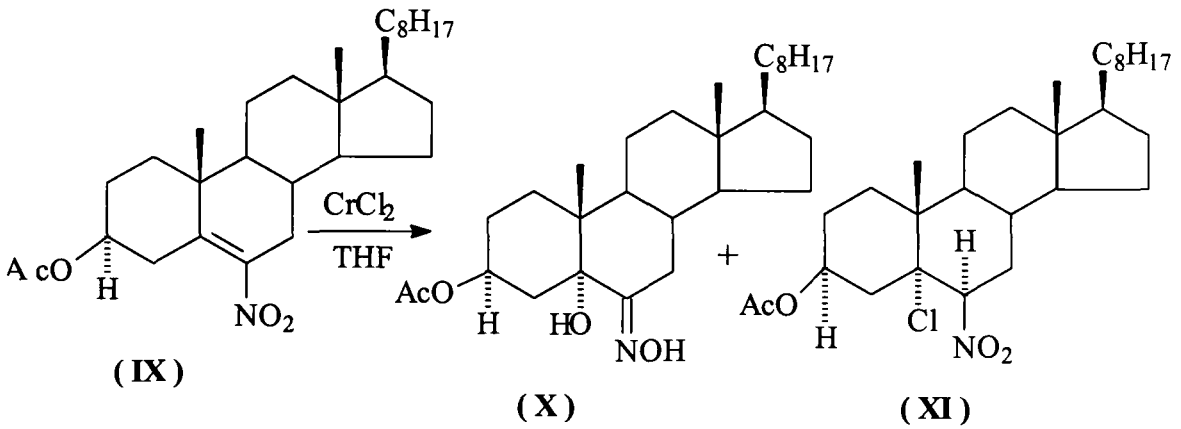
Kaye et.al.[2] treated 3β-chloro-6-nitrocholest-5-ene (I) with zinc and acetic acid at 100° to obtain 3α, 5-cyclo-5α-cholestan-6-one (II) and 3β-chloro-5α-cholestan-6-one oxime (III). 2-Nitro-Δ^2-cholestene (IV) under similar conditions provided 5α-cholestan-2-one[3] (V).



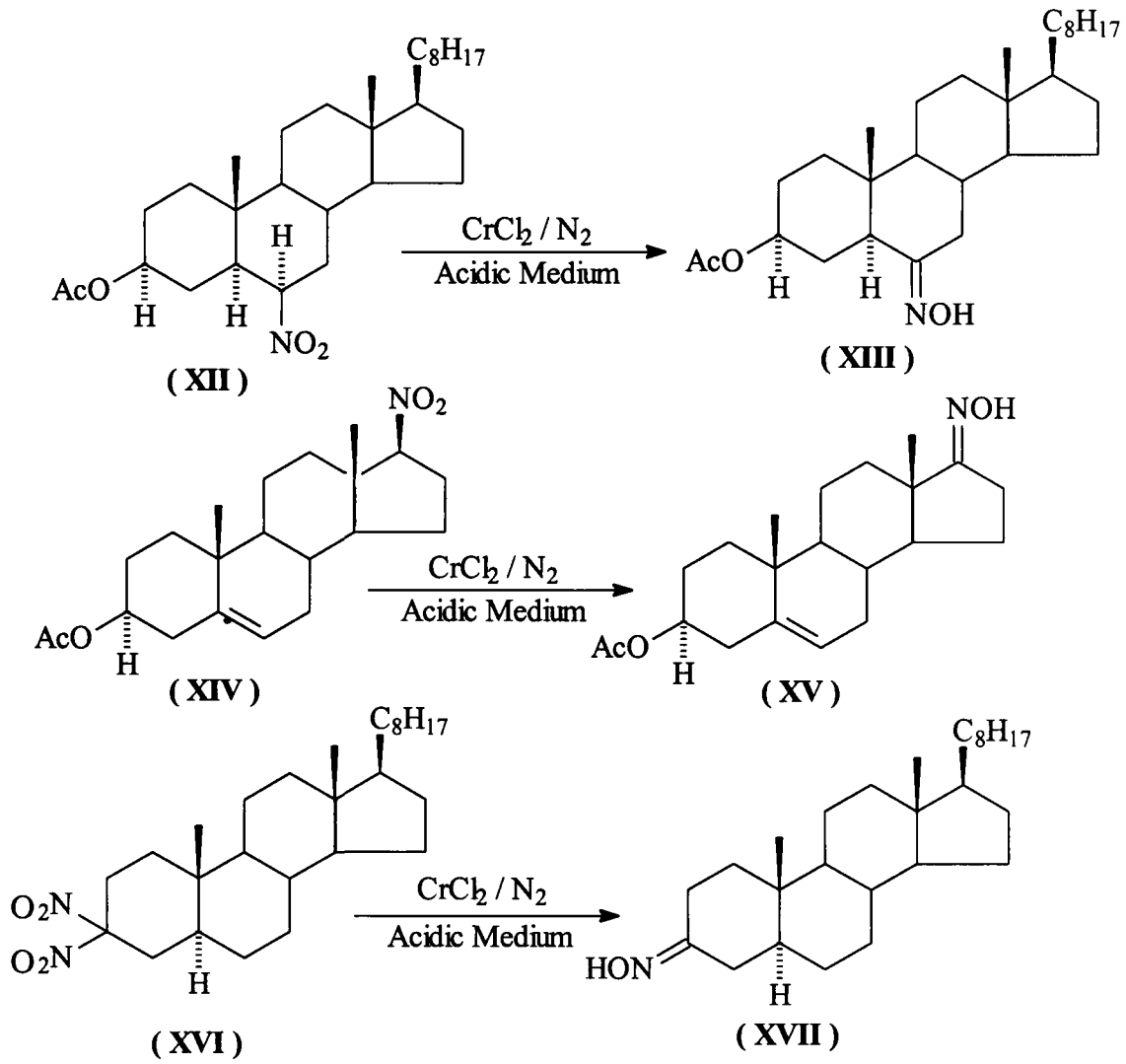
Komeichi et.al.[4] treated 3β-acetoxy-5-chloro-6-oximino-5α-cholestane (VI) with various nucleophiles in dichloromethane to furnish 5α-substituted oxime (VII) alongwith 3β-acetoxycholest-4-en-6-one oxime (VIII).



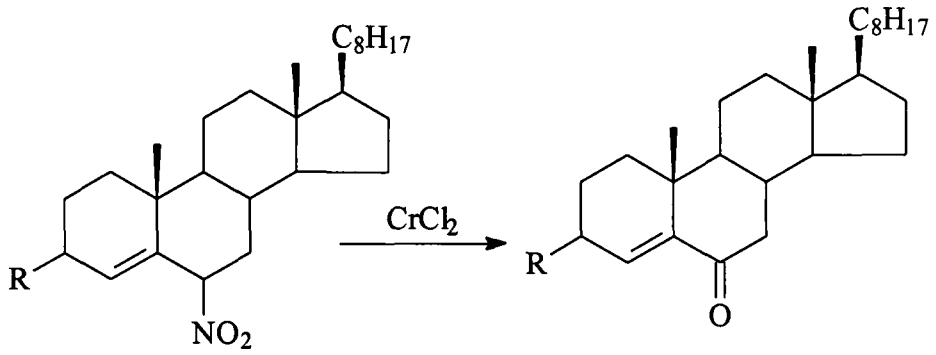
Hanson and Premuzic[5] refluxed a mixture of 3β-acetoxy-6-nitrocholest-5-ene (IX) and chromous chloride to give the 3β-acetoxy-5α-hydroxycholestan-6-one oxime (X) and 3β-acetoxy-5-chloro-6β-nitro-5α-cholestane (XI).



Hanson and Organ[6] carried out the reduction of 3β-acetoxy-6β-nitro-5α-cholestane (XII) under nitrogen gas with acidic chromium (II) chloride to afford 3β-acetoxy-6-oximino-5α-cholestane (XIII). 3β-Acetoxy-17β-nitrocholest-5-ene (XIV) under similar reaction conditions provided oxime (XV) whereas 3,3-dinitro-5α-cholestane (XVI) was reduced to 3-oxime (XVII).



In contrast to the above cases chromium (II) chloride reduction of β,γ-unsaturated nitro steroids such as 3β-acetoxy-6-nitrocholest-4-ene (XVIII) and 3β-hydroxy-6-nitrocholest-4-ene (XIX) afforded the ketones, 3β-acetoxy-4-en-6-one (XX) and cholest-4-en-6-one (XXI), rather than the oximes. Reduction of both 6α and 6β-nitrocholest-4-en-3-ones (XXII) provided 3,6-dione[6] (XXIII).

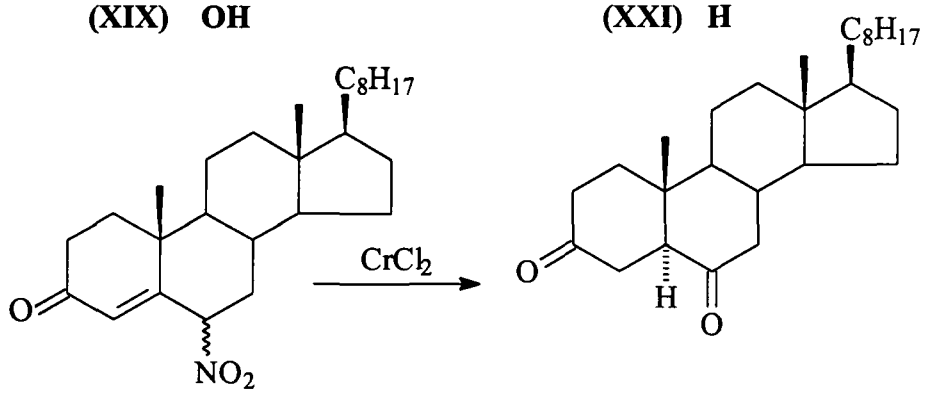


R

(XVIII) OAc
(XIX) OH

R

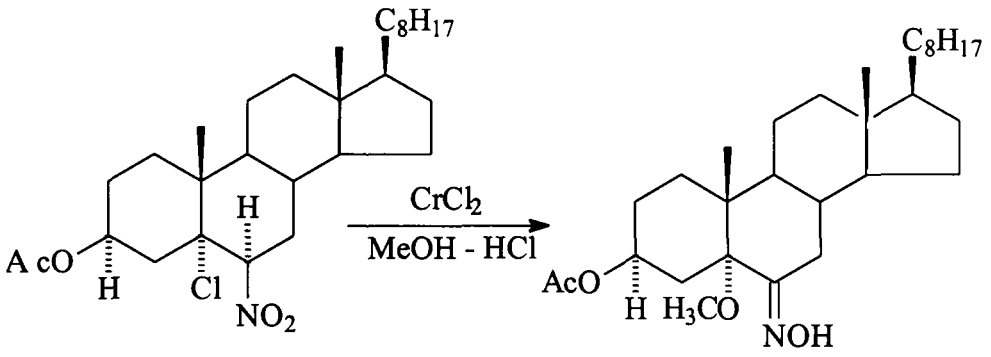
(XX) OAc
(XXI) H



(XXII)

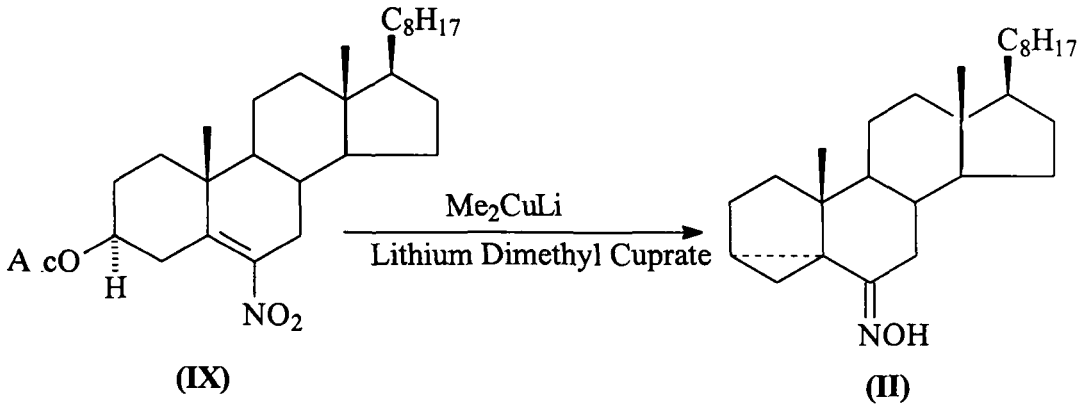
(XXIII)

Hassner and Heathcock[7] treated 3β-acetoxy-5-chloro-6β-nitro-5α-cholestane (XXIV) with chromous chloride in methanolic hydrochloric acid to obtain 3β-acetoxy-5α-methoxycholestan-6-one oxime (XXV).



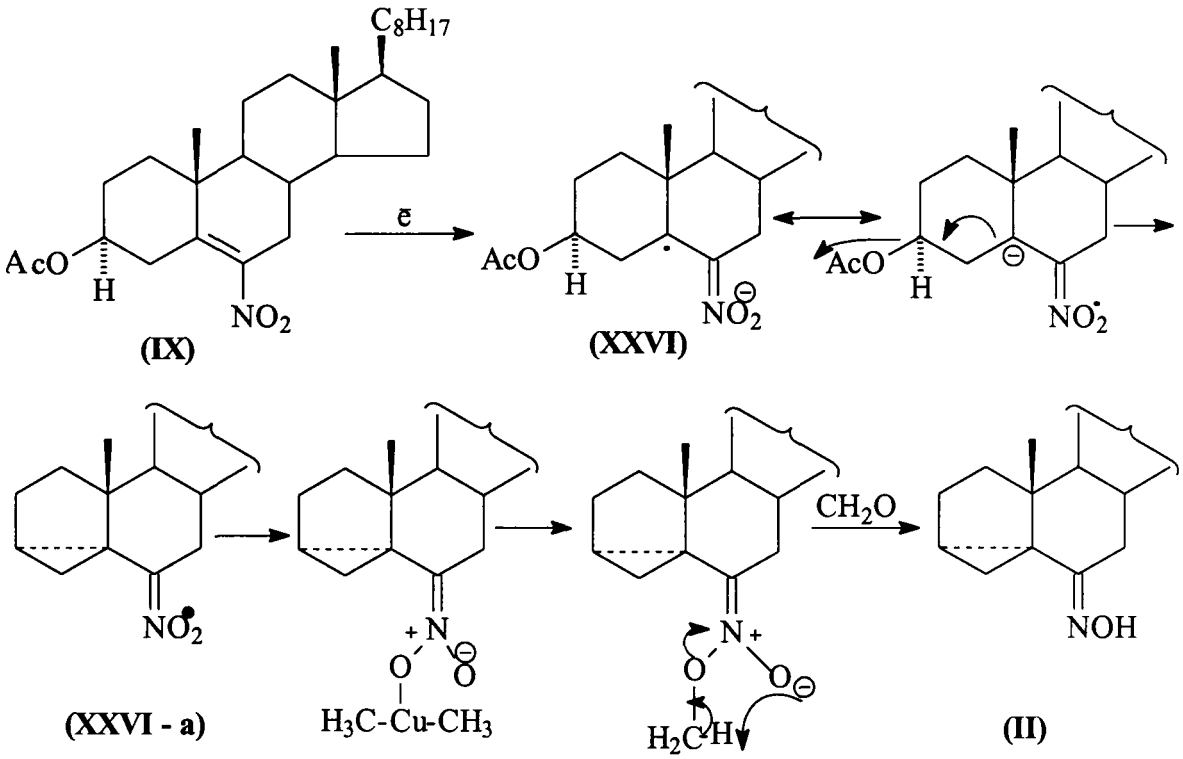
(XXIV) **(XXV)**

Stiver and Yates[8] carried out the reaction of 3β-acetoxy-6-nitrocholest-5-ene (IX) with an excess of lithium dimethylcuprate to give 3α, 5-cyclo-5α-cholestan-6-one oxime (II).



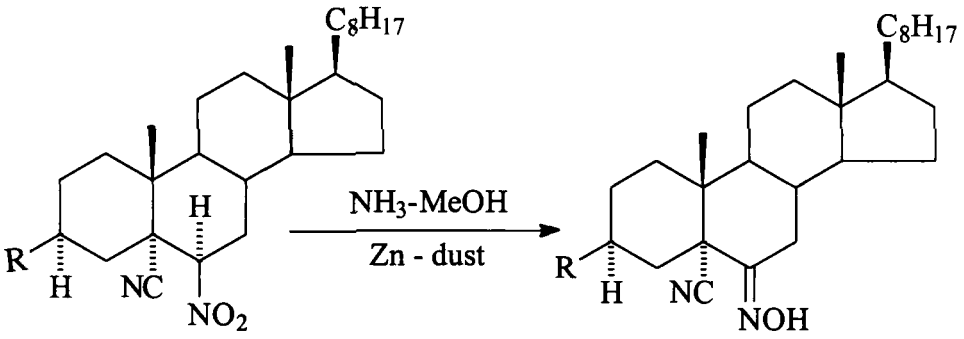
(IX) **(II)**

The mechanism for the formation of product (II) from (IX) was proposed as shown in scheme-1. It was suggested that the formation of (II) from (IX) is initiated by one electron transfer from lithium dimethyl cuprate to give radical anion (XXVI). An internal displacement of the acetoxy group in (XXVI) would be the 3α, 5α-cyclo species (XXVI - a). The displacement of acetate ion may be facilitated by complexation with lithium or copper species.



Scheme - 1

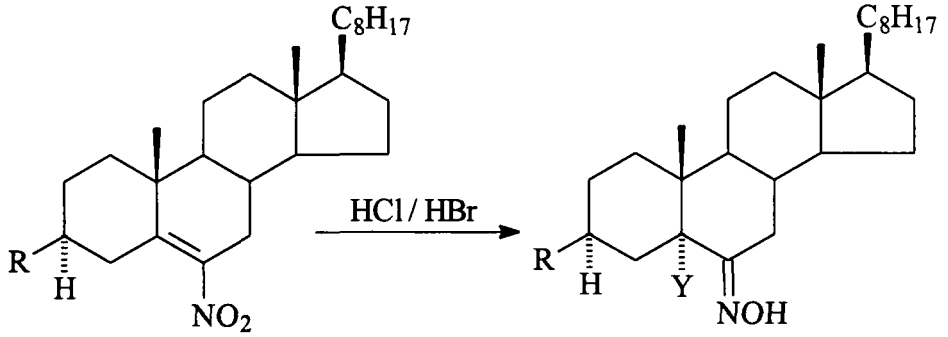
Nitrocyanides (XXVII a-d) were converted with ammonia, methanol and zinc dust to the corresponding cyano-oximes[9] (XXVIII a-c,VII).



	R
(XXVII-a)	H
(XXVII-b)	OH
(XXVII-c)	Cl
(XXVII-d)	OAc

	R
(XXVIII-a)	H
(XXVIII-b)	OH
(XXVIII-c)	Cl
(VII)	OAc

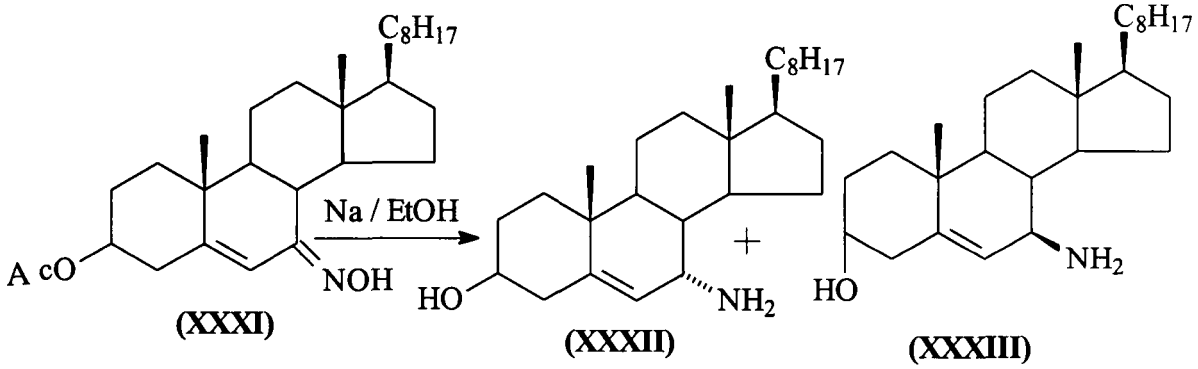
Yoshihisa et.al.[10] reported that the treatment of 6-nitrocholest-5-enes (I, IX and XXIX) with dry hydrogen chloride or bromide afforded hitherto unaccessible 5α-chloro or 5α-bromo-6-oximino-5α-cholestanes (XXX a-d) in good yield.



	R
(I)	Cl
(IX)	OAc
(XXIX)	H

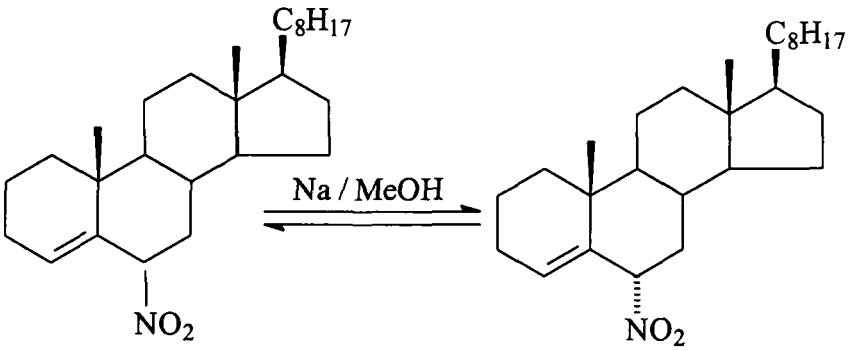
	R	Y
(XXX-a)	Cl	Cl
(XXX-b)	OAc	Cl
(XXX-c)	H	Cl
(XXX-d)	OAc	Br

Reduction of 3β-acetoxycholest-5-en-7-one oxime (XXXI) with sodium metal and ethyl alcohol gave 7α and 7β-amino-cholesterols[11] (XXXII and XXXIII).



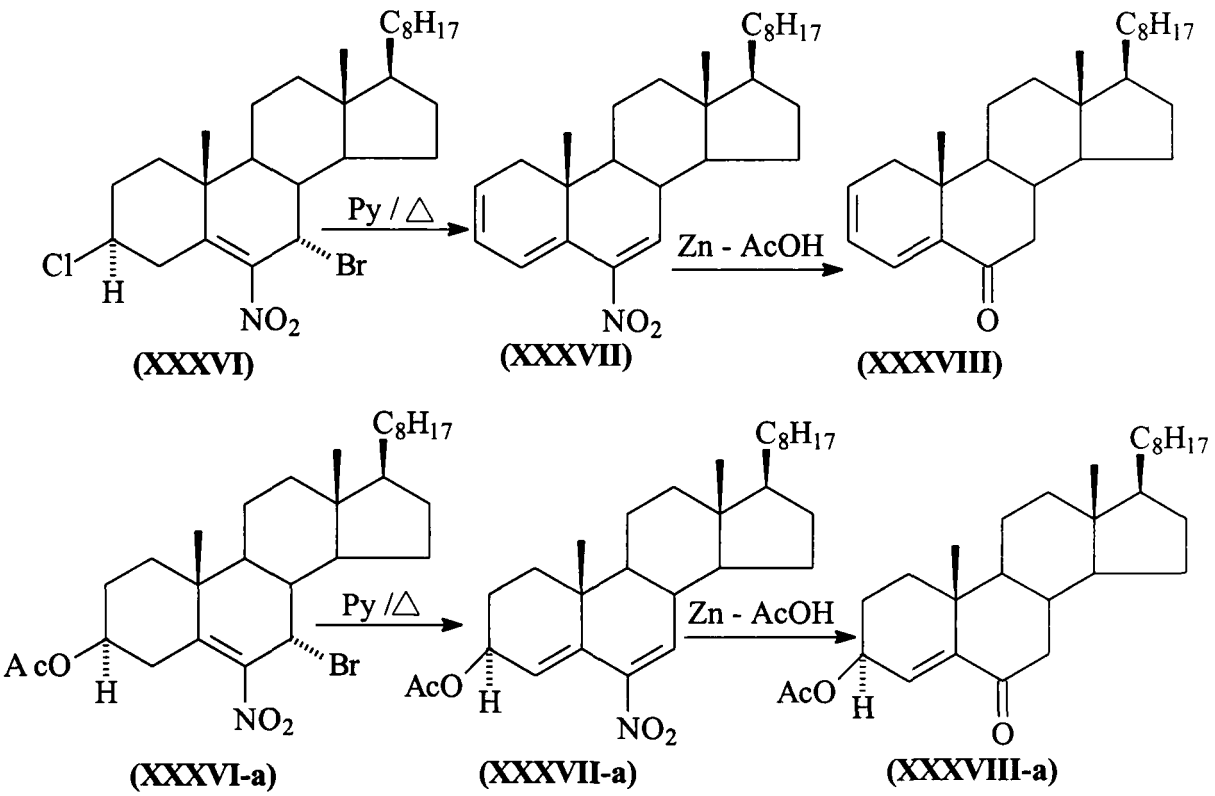
(XXXI) (XXXII) (XXXIII)

Pinhey et.al.[12] reported that the treatment of 6β-nitrocholest-4-en (XXXIV) with catalytic amount of sodium methoxide in methanol gave an equilibrium mixture which contained the starting material (XXXIV) and the 6α-epimer (XXXV) in a 1:1 ratio. According to them 6α-nitro steroid is thermodynamically more stable that its 6β-epimer.

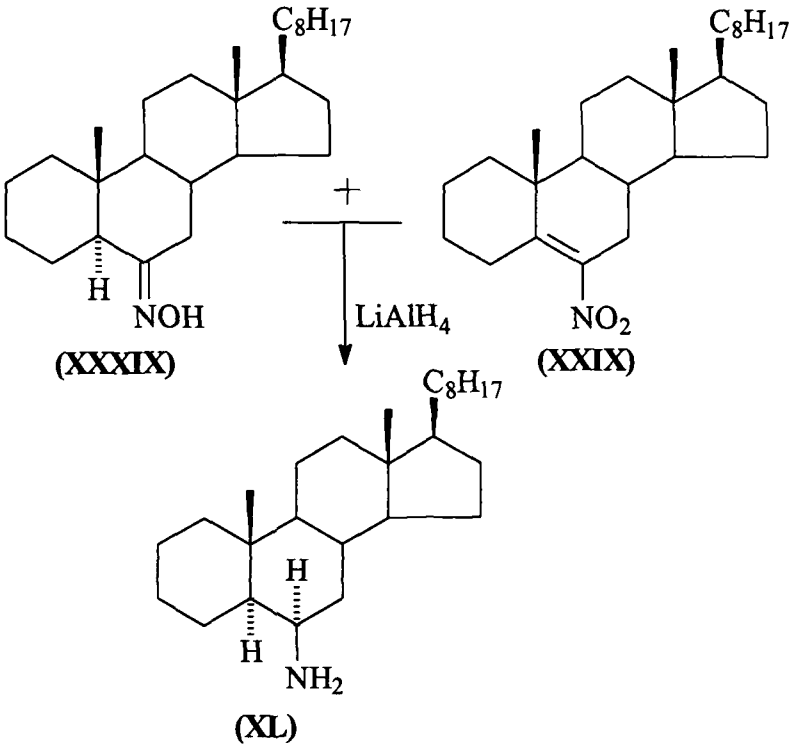


(XXXIV) **(XXXV)**

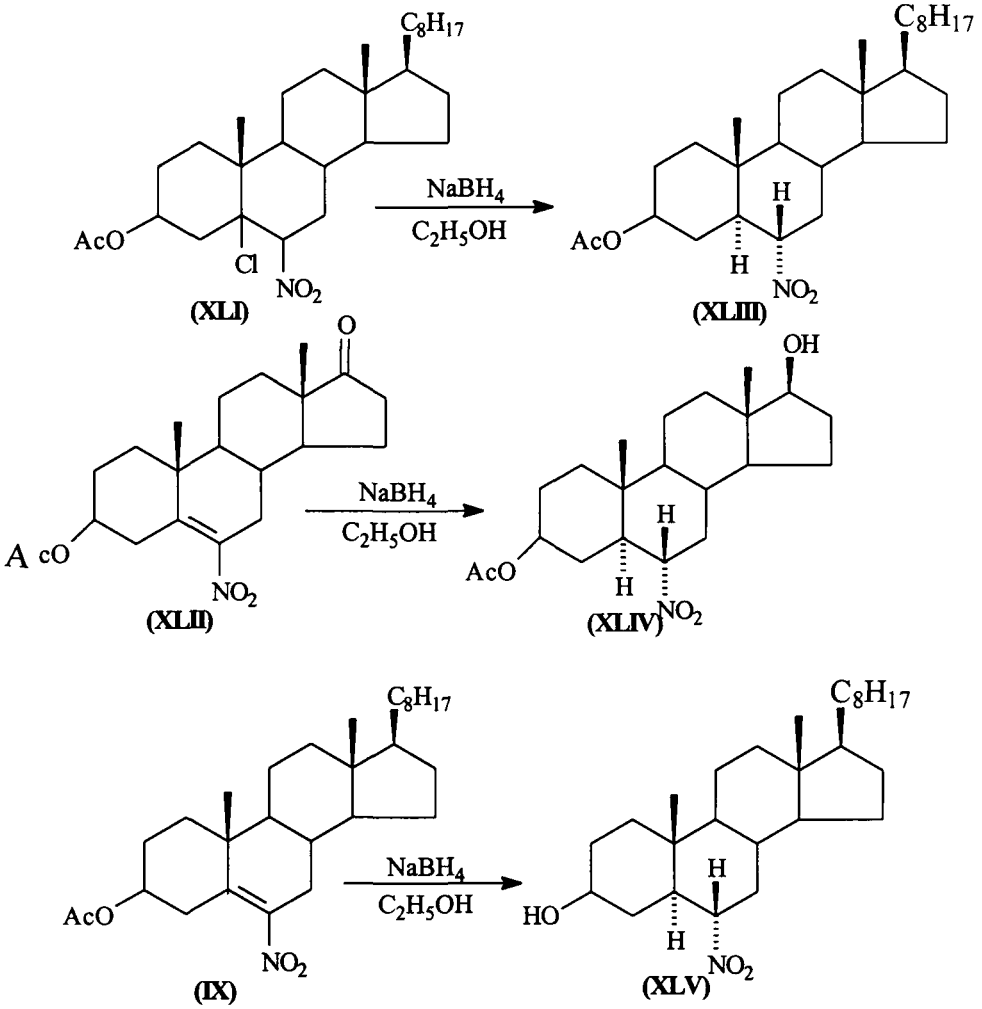
Treatment of (XXXVI and XXXVI – a) with pyridine under reflux condition provided the dehydrobrominated compounds (XXXVII and XXXVII - a) which on zinc acetic acid reduction resulted in the formation of respective ketones (XXXVIII and XXXVIII - a)[13].



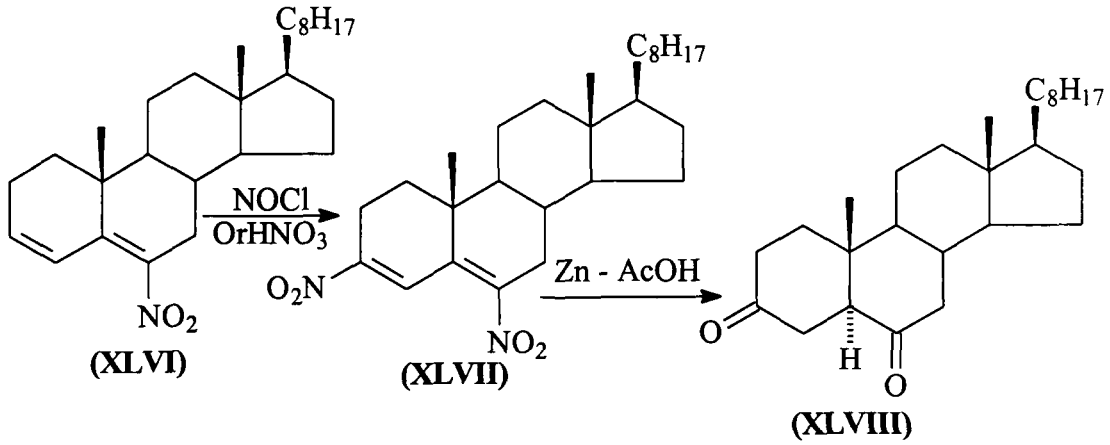
Shoppee et.al.[14] reported that cholestan-6β-yl amine (X) was prepared by reduction of cholestan-6-one oxime (XXXIX) and nitrocholest-5-ene (XXIX) with lithium aluminium hydride.



Steroidal nitro compounds (XLI, XLII - IX) have been reported to give 6α-nitrosteroids (XLIII - XLV), on reduction with sodium borohydride in ethanol[7].

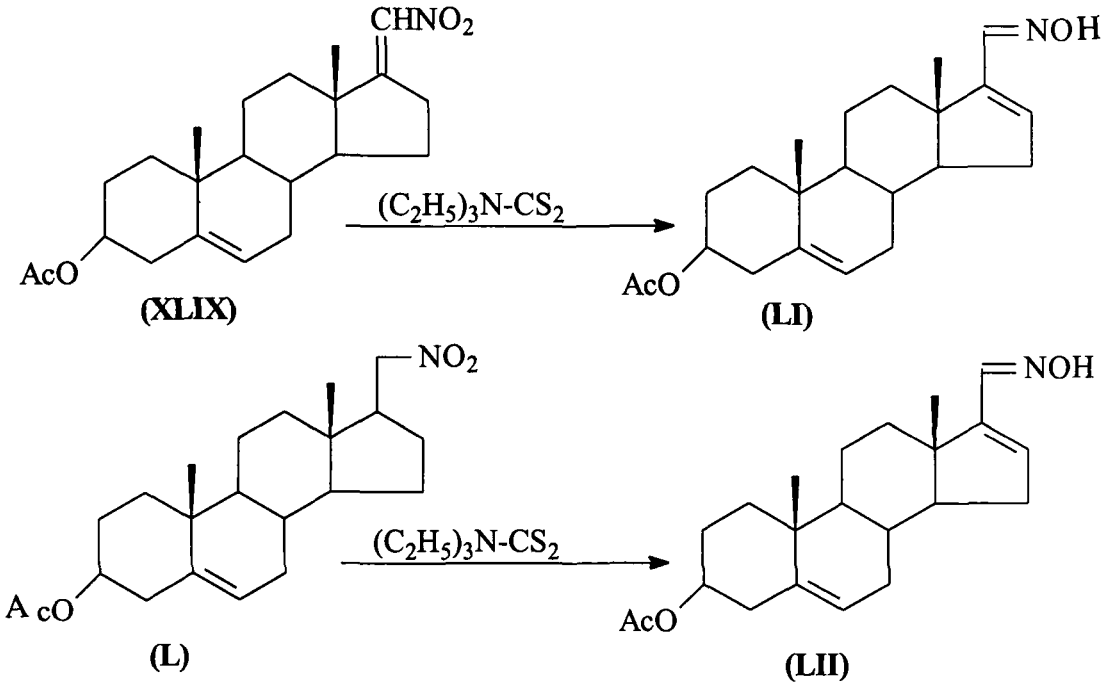


3, 6-Dinitrocholesta-3, 5-diene (XLVII) was obtained either by the reaction of nitrosyl chloride or nitric acid with 6-nitrocholesta-3, 5-diene (XLVI). Zinc acetic reduction of (XLVII) gave 3, 6-dione[15] (XLVIII).



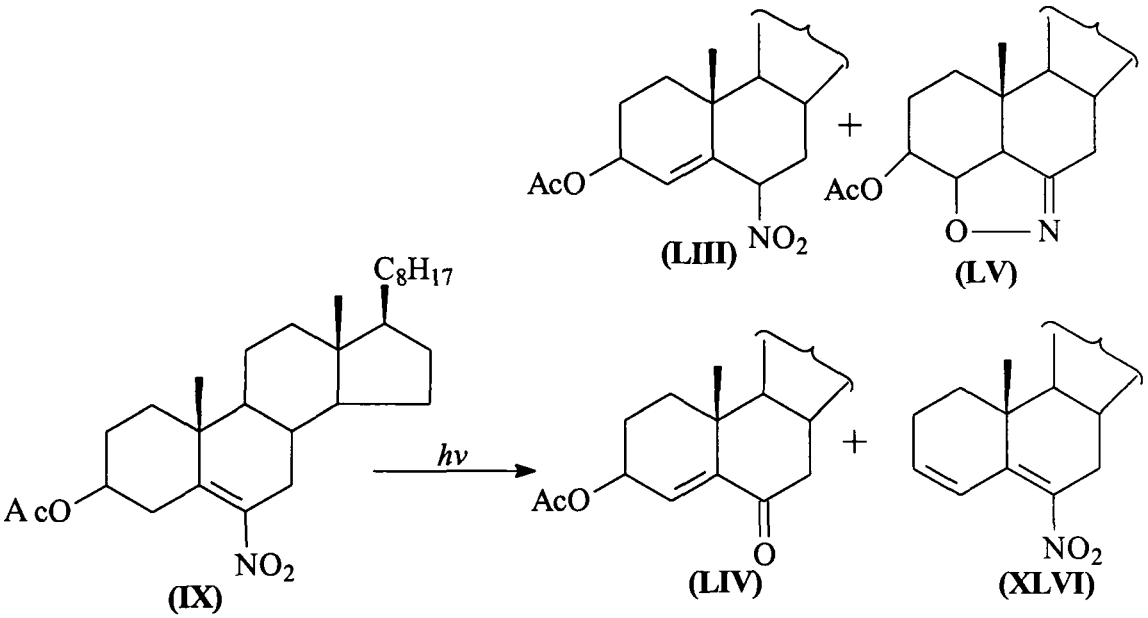
(XLVI) (XLVII) (XLVIII)

Barton et.al.[16] reported the reduction of nitro compounds (XLIX and L) to corresponding oximes (LI and LII) when treated with trimethylamine and carbon disulfide.

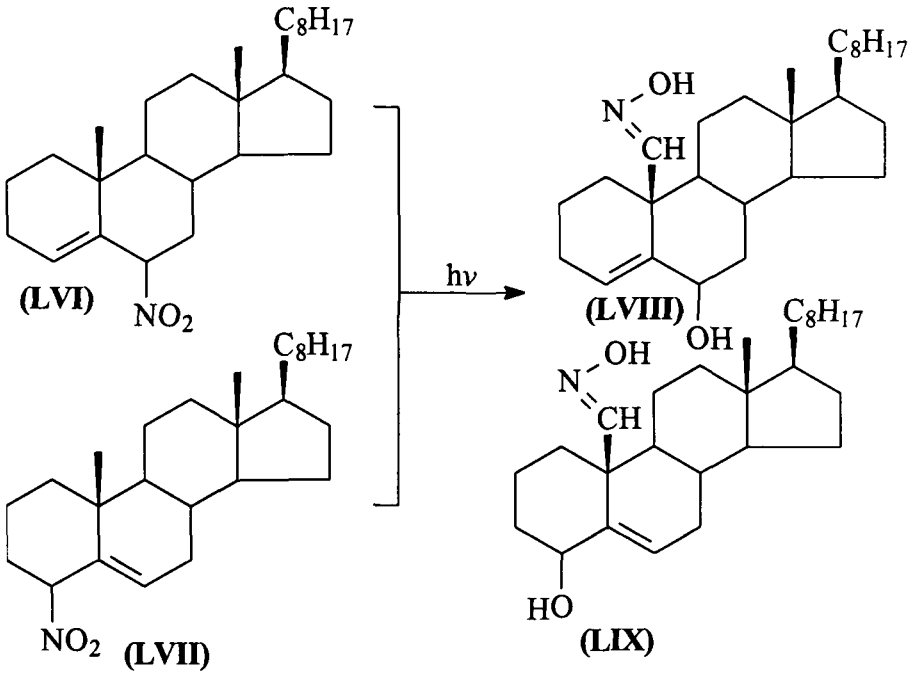


(XLIX) (LI) (L) (LII)

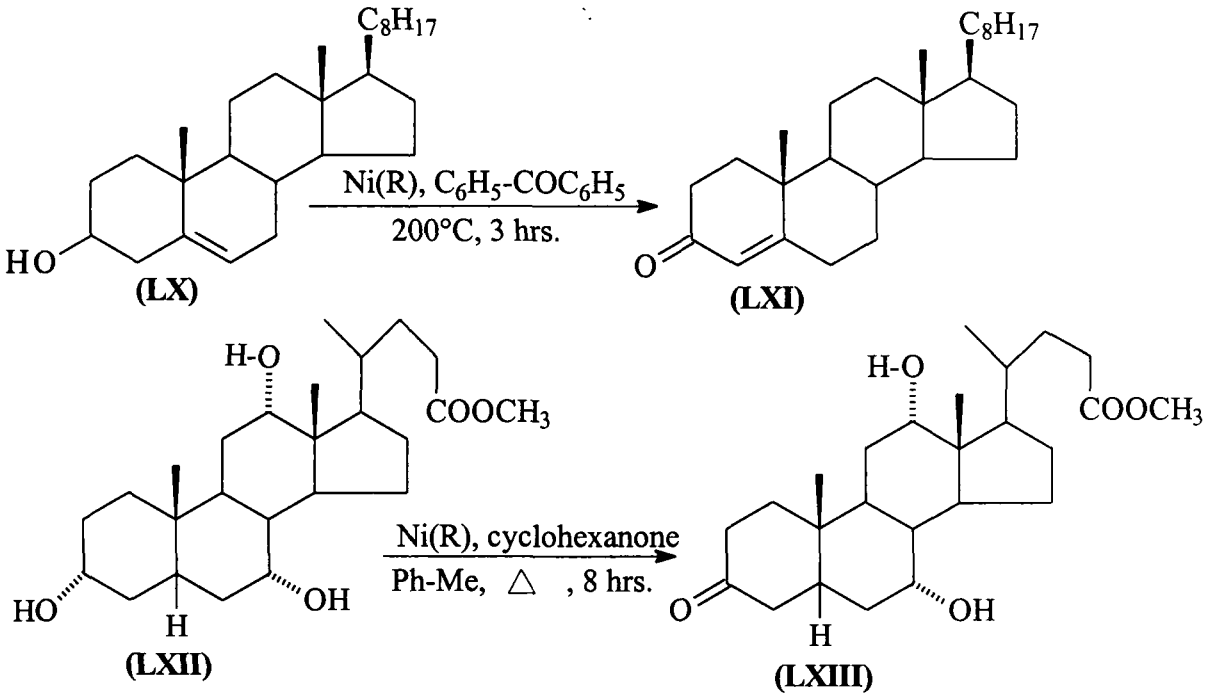
It has been reported[16a,b,c] that the irradiation of 3β-acetoxy-6-nitrocholest-5-ene (IX) in hexane gave 6β-nitrocholest-4-en-3β-yl acetate (LIII) along with other products (LIV, LV and XLVI).



Photolysis of both 6β-nitrocholest-4-ene (LVI) and 4β-nitrocholest-5-ene (LVII) gave a mixture of hydroxyimino cholesterols (LVIII and LIX). This was reported to arise from a photochemical nitro-nitrosooxy rearrangement followed by Barton reaction[17].

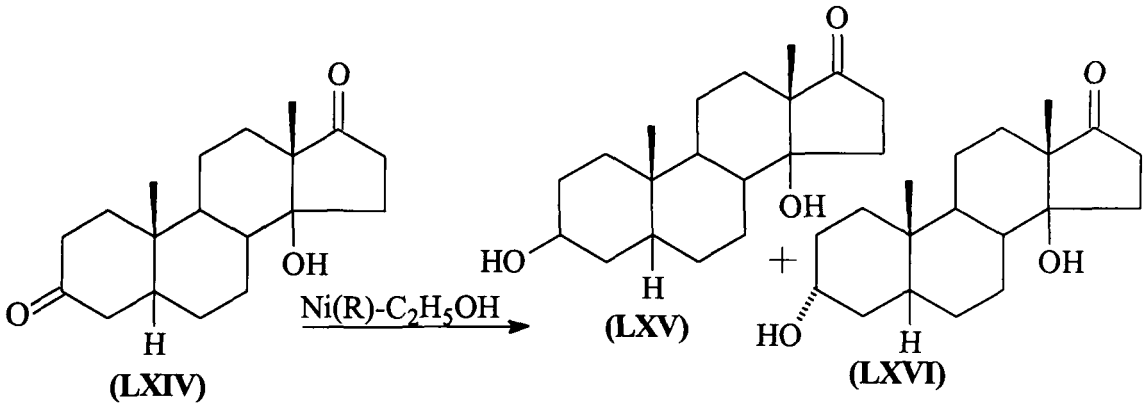


Raney nickel was used for the dehydrogenation of sterols by Foster et.al[18] when cholesterol (LX) was treated at 260° for 3 hours with Raney nickel and benzophenone gave cholest-4-en-3-one (LXI). Similarly trihydroxy compound (LXII) gave 3-ketone (LXIII) when treated with Raney nickel and cyclohexanone in toluene.

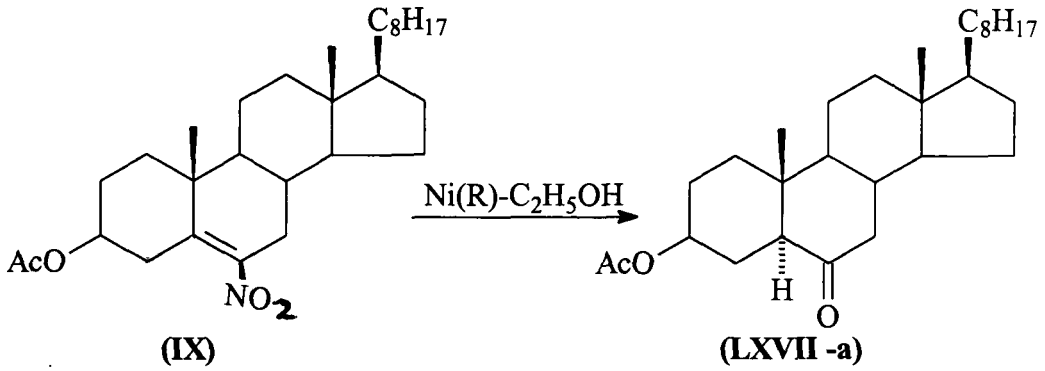


(LX) **(LXI)** **(LXII)** **(LXIII)**

Reduction of 14β-hydroxy-5β, 14β-estran-3, 17-dione (LXIV) in 95% aqueous ethanol containing Raney nickel gave 3β, 14β-dihydroxy-5β, 14-estran-17-one (LXV) and 3α, 14β-dihydroxy-5β,14β-estran-17-one[19] (LXVI). It has been shown that 3β-acetoxy-6-nitrocholest-5-ene (IX -a) under the same reaction condition provided 3β-acetoxy-5α-cholestan-6-one[20] (LXVII -a).

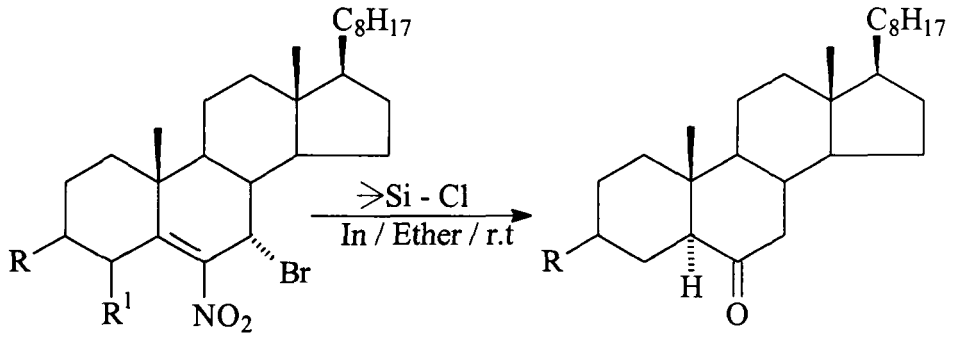


(LXIV) (LXV) (LXVI)



(IX) (LXVII -a)

Recently the reduction of bromo nitrosteroidals (LXVIII a - d) to steroidal 6-ketones (LXVII a – d) by chlorotrimethyl silane[21] is reported.

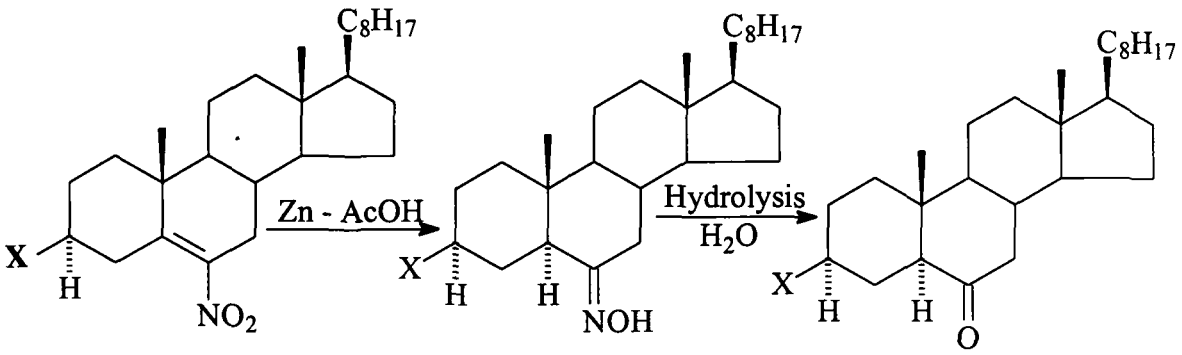


	R	R[1]
(LXVIII-a)	OAc	H
(LXVIII-b)	Cl	H
(LXVIII-c)	H	Br

	R
(LXVII-a)	OAc
(LXVII-b)	Cl
(LXVII-c)	H

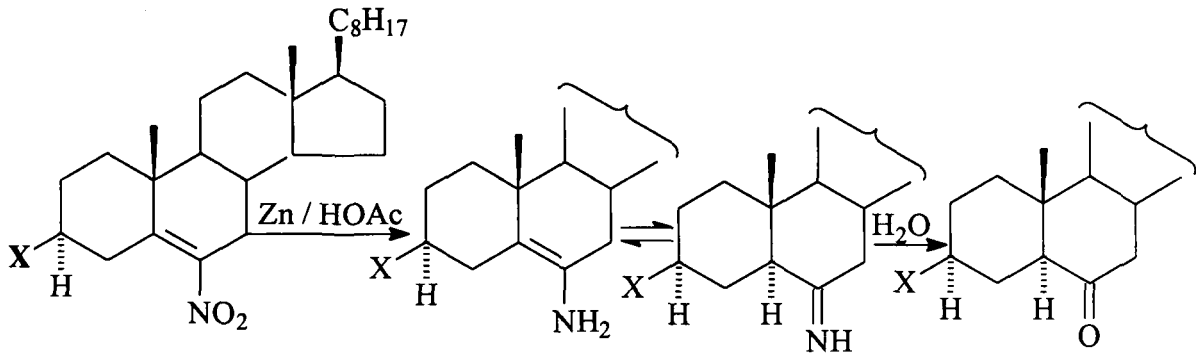
DISCUSSION

Previous work from these laboratories described the conversion of steroidal vinyl nitro compounds into oximes followed by reductive hydrolysis to corresponding steroidal ketones by Zn-acetic acid. Kaye et al.[2] suggested that the oximes are the direct precursors of the ketones in these reduction.



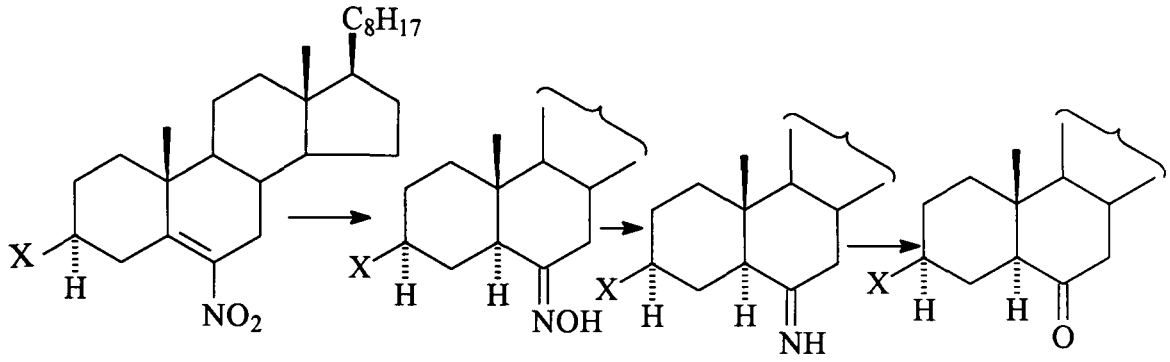
X = Cl, OAc, OH and H

This is in contrast to the previously described mechanism in which an imine[22] was considered to be the precursor of ketone.



X = Cl, OAc, OH

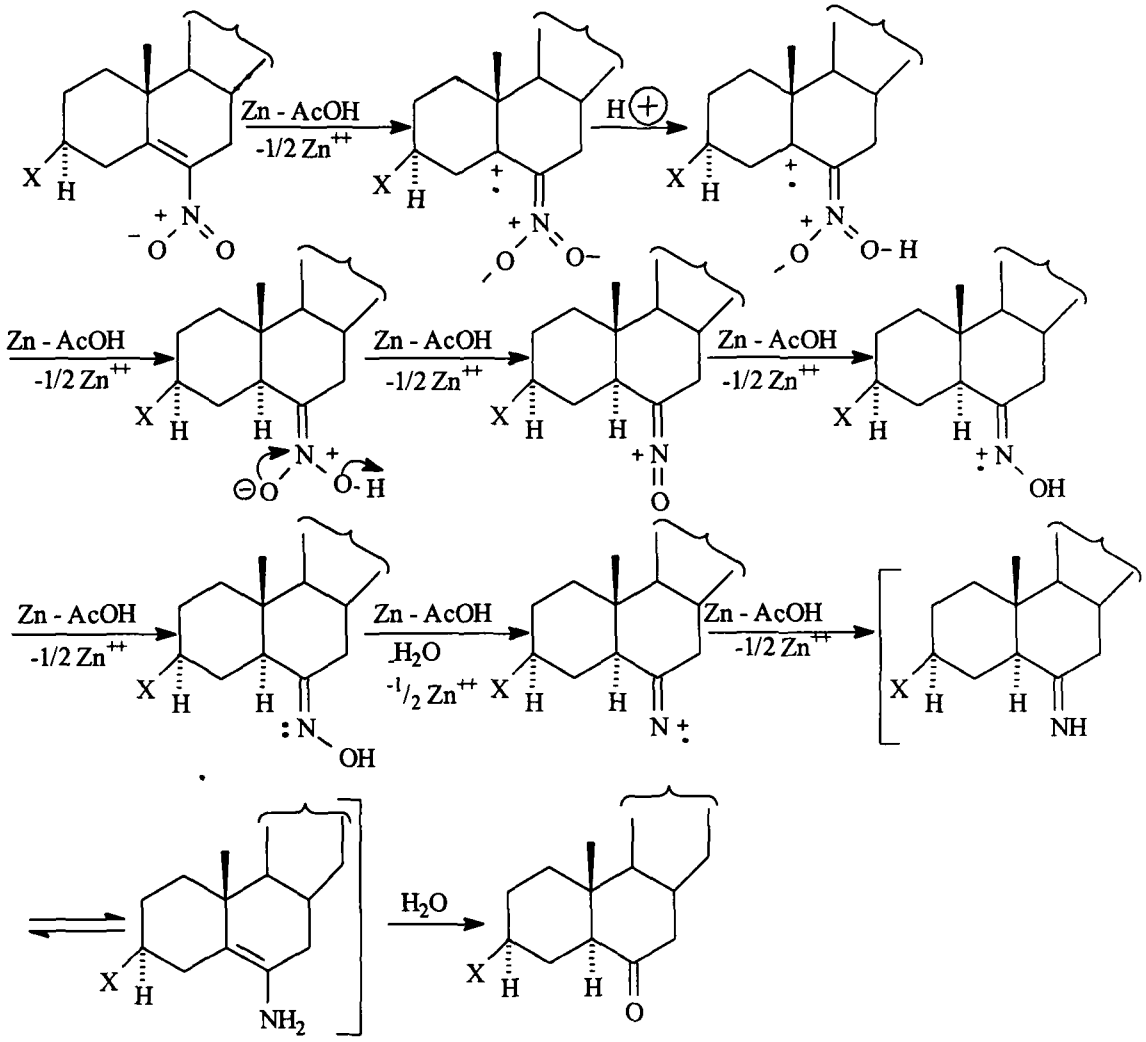
It has been reported that if steroidal ketoxime was heated with AcOH-water mixture, steroidal oxime remained unchanged and no ketone was obtained as suggested by Kaye et al.[2] The sensitivity of a steroidal ketoxime towards zinc-acetic acid – water mixture and not towards acetic acid and water strongly suggests that the oxime is further reduced presumably to an imine (A) and the latter undergoes hydrolytic cleavage to give the ketone[23].



(A)

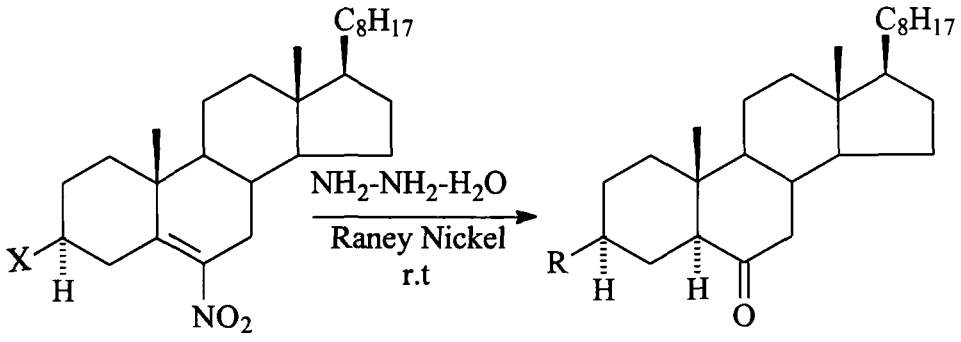
X = Cl, OAc, OH, H

A mechanism explaining the role of zinc (a reducing metal : zn → ½zn^{++}, + 0.761 volt at 25°)[24a,b] as electron donar[25] has been written for total transformation of vinyl nitro steroids to steroidal ketones.



In continuation to work related to reduction of vinyl nitrosteroids with Zn-AcOH to steroidal ketones. We have tried the above transformation with hydrazine-hydrate-Ni (R)[26]. As a matter of fact this attempt was made earlier[27] also but detail critical study revealed remarkable difference in reaction conditions, yield of the steroidal ketones needed for variety of steroidal reactions.

we subjected 3β-acetoxy-6-nitrocholest-5-ene (IX), 3β-chloro-6-nitrocholest-5-ene (I), 6-nitrocholest-5-ene (XXIX) and 3β-hydroxy-6-nitrocholest-5-ene (LXIX) to hydrazine-hydrate reduction in the presence of Raney Nickel as catalyst. It has been observed that the transformation of steroidal vinyl nitro compounds occurred at room temperature and yield of the corresponding ketones (LXVII a-d) was much higher as compared to zn-AcOH reduction[23].

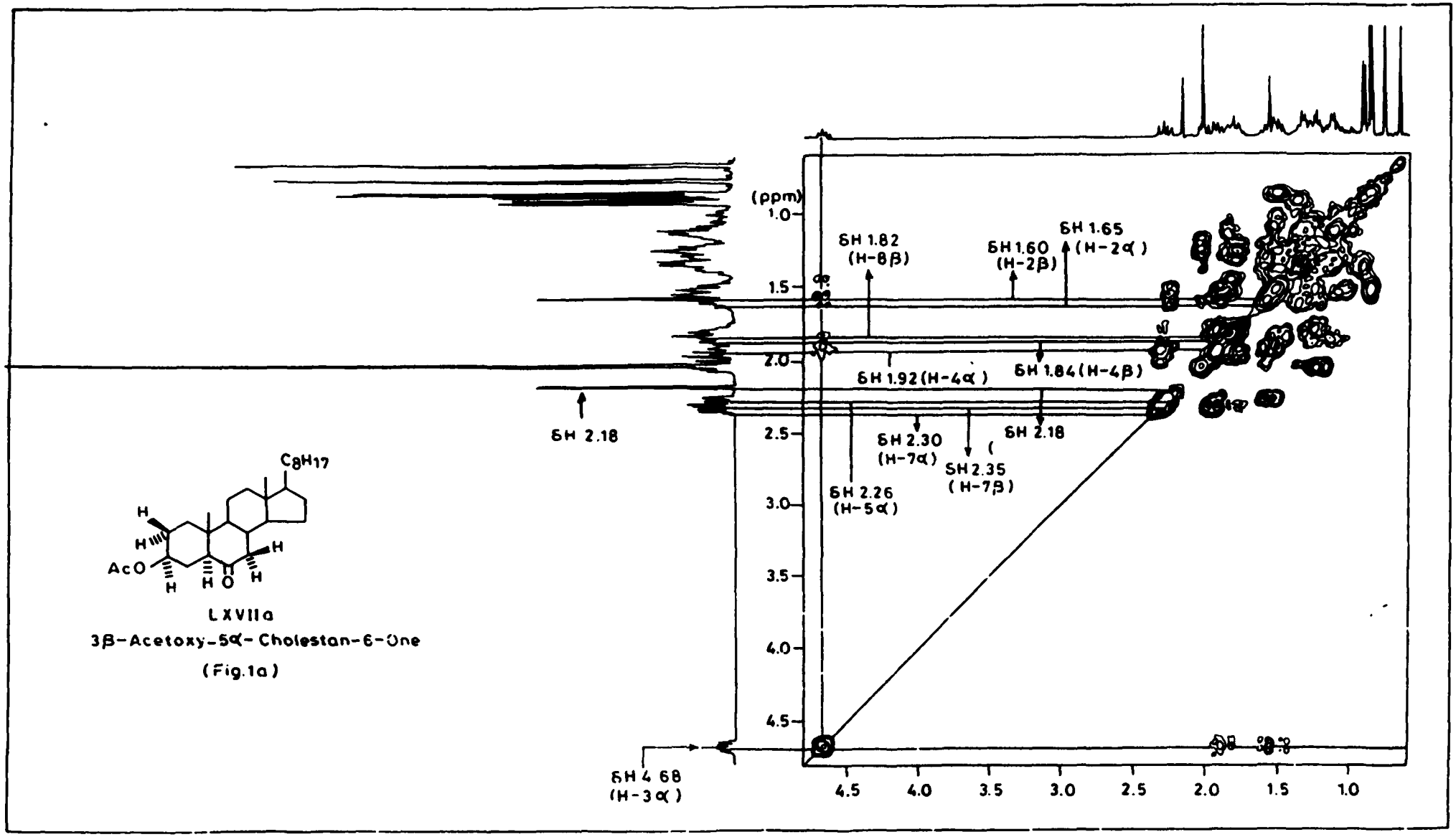


	X
(IX)	OAc
(I)	Cl
(XXIX)	H
(LXIX)	OH

	X
(LXVII-a)	OAc
(LXVII-b)	Cl
(LXVII -c)	H
(LXVII-d)	OH

Hydrazine-Hydrate Reduction of 3β-acetoxy-6-nitrocholest 5-ene (IX) catalysed by Raney Nickel : 3β-Acetoxy-5α-cholestan-6-one (LXVII-a)

3β-Acetoxy-6-nitrocholest-5-ene (IX) in ethanol is treated with hydrazine hydrate in the presence of Raney nickel as per procedure of First

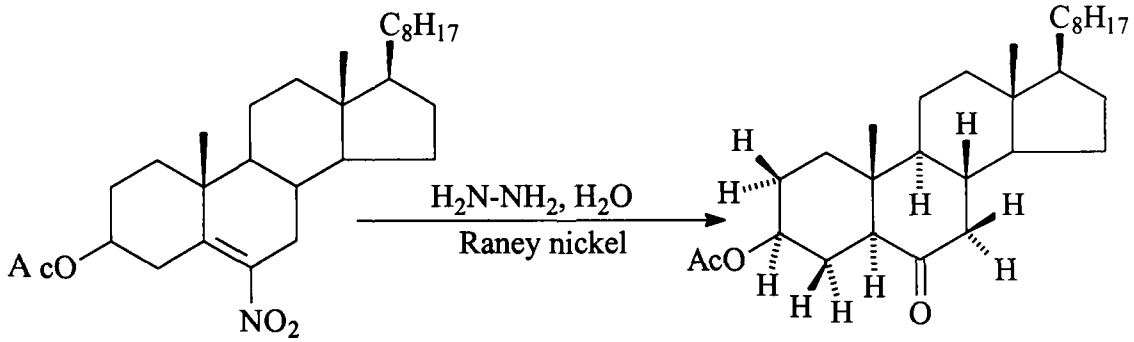


LXVIIa

3β-Acetoxy-5α-Cholestan-6-One

(Fig.1a)

and Moore[26] at room temperature. The reaction mixture after usual work up and column chromatography over silica gel provided crystalline product, m.p. 128° (reported[28], m.p.. 127).

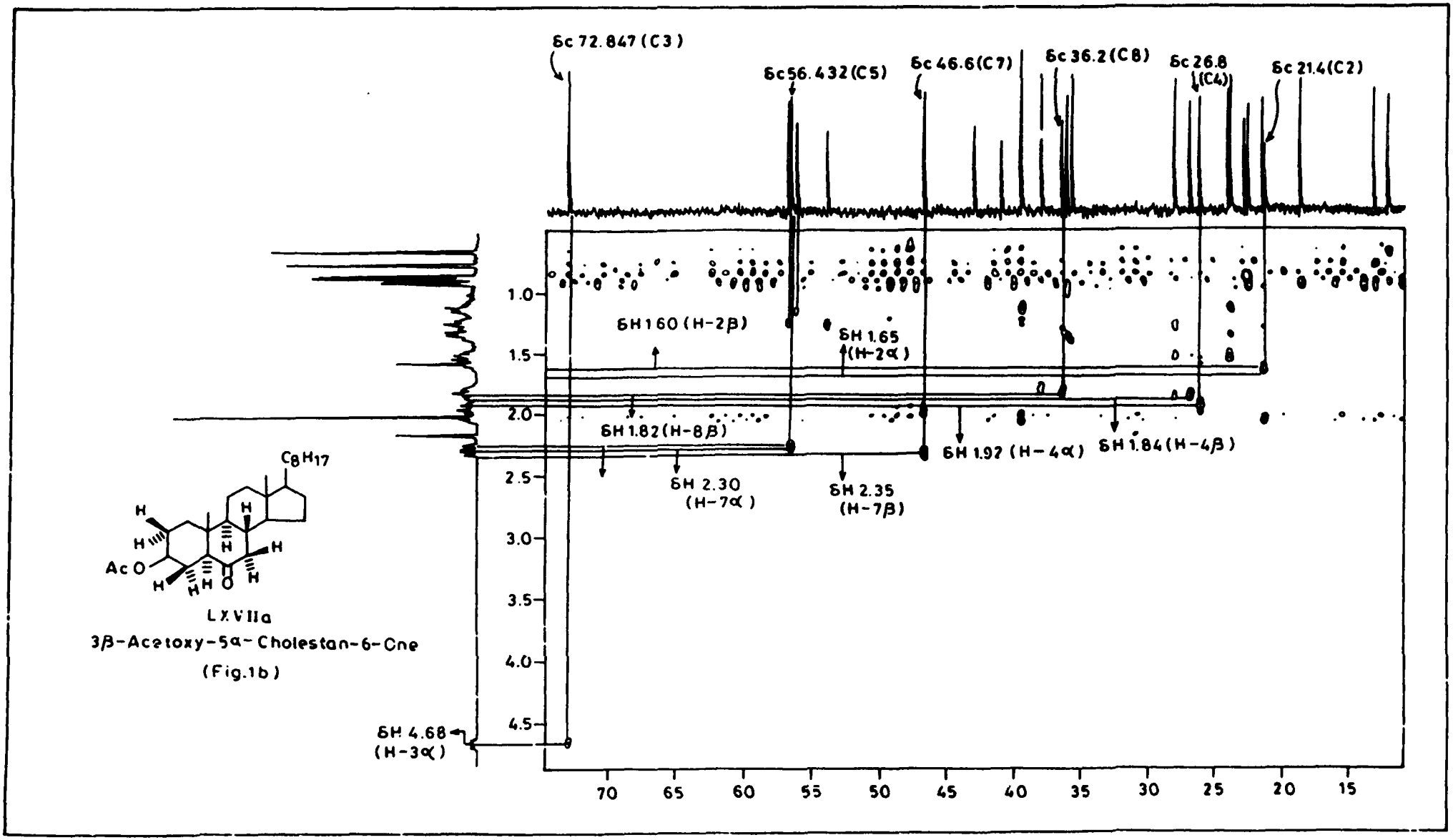


(IX) **(LXVII-a)**

Compound, m.p. 128° as 3β-acetoxy-5α-cholestan-6-one (LXVII-a):

The compound (LXVII-a), m.p. 128° (reported[28], m.p. 127°) was analysed $C_{29}H_{48}O_3$. In the IR spectrum bands were found at 1735 (-O-CO-CH_3), 1710 (C=O), 1210, 1035 cm^{-1} (C-O).

^1H-^1H-NMR homonuclear cosy spectrum of 3β-acetoxy-5α-cholestan-6-one (LXVII – a) (Fig. 1a) :



LXVIIa

3β-Acetoxy-5α-Cholestan-6-One

(Fig.1b)

The ^{1}H-^{1}H-NMR cosy spectrum of 3β-acetoxy-5α-cholestan-6-one (LXVII – a) (Fig. 1a) gave contour on diagonal at δ 4.68 (H-3α) which is coupled by H-4α (δ 1.92), H-4β (δ 1.84), H-2α (δ 1.65) and H-2β (δ 1.60), H-5α(δ 2.26)appeared as double doublet (Jae=4.5 Hz, Jaa=12 Hz) coupled by H-4α (δ 1.92) and H-4β (δ 1.84). A singlet δ 2.18 in the ^{1}H-^{1}H-NMR cosy spectrum is assigned to protons of acetate methyl. The contour at δ 2.18 on diagonal has no diagonal cross over multiplet. The H-7β appeared as double doublet at δ 2.35 (J=4.5 Hz and 12 Hz). This proton is coupled by H-7α (δ 2.30) and H-8β at (δ 1.82).

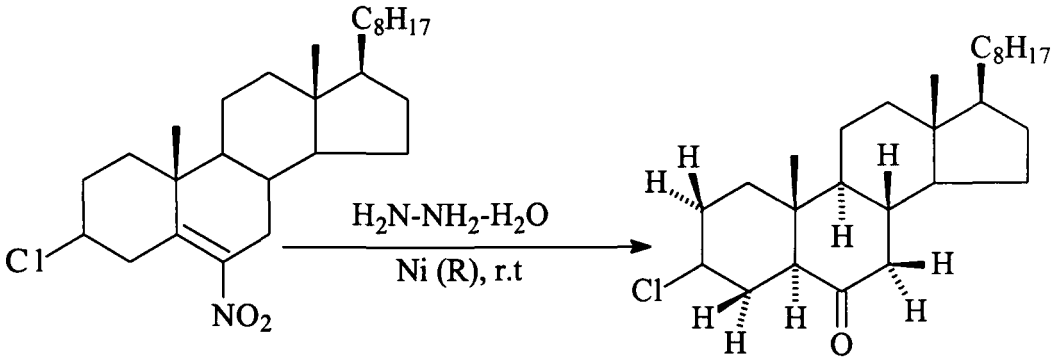
^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β-acetoxy-5α-cholestan-6-one (LXVII – a) (Fig. 1b) :

^{1}H-^{13}C-NMR cosy spectrum of 3β-acetoxy-5α-cholestan-6-one (LXVII-a) (Fig. 1b) correlates the δ (chemical shift) of protons with their ^{13}C values. δ 4.68 (H-3α) is correlated to δc 72.847 (C3), δ 2.26 (H-5α) to δc 56.432 (C5), δ 2.35 (H-7β) and δ 2.30 (H-7α) to δc 46.6 (C7).

The mass spectrum of 3β-acetoxy-5α-cholestan-6-one (LXVII-a) gave molecular ion peak at m/z 444 ($M^{+\cdot}$) followed by peaks at m/z 384 ($M^{+\cdot}$ -CH_3COOH), m/z 366 (m/z 384 – H_2O) and other lower mass peaks.

Hydrazine-Hydrate Reduction of 3β-chloro-6-nitrocholest-5-ene (I):

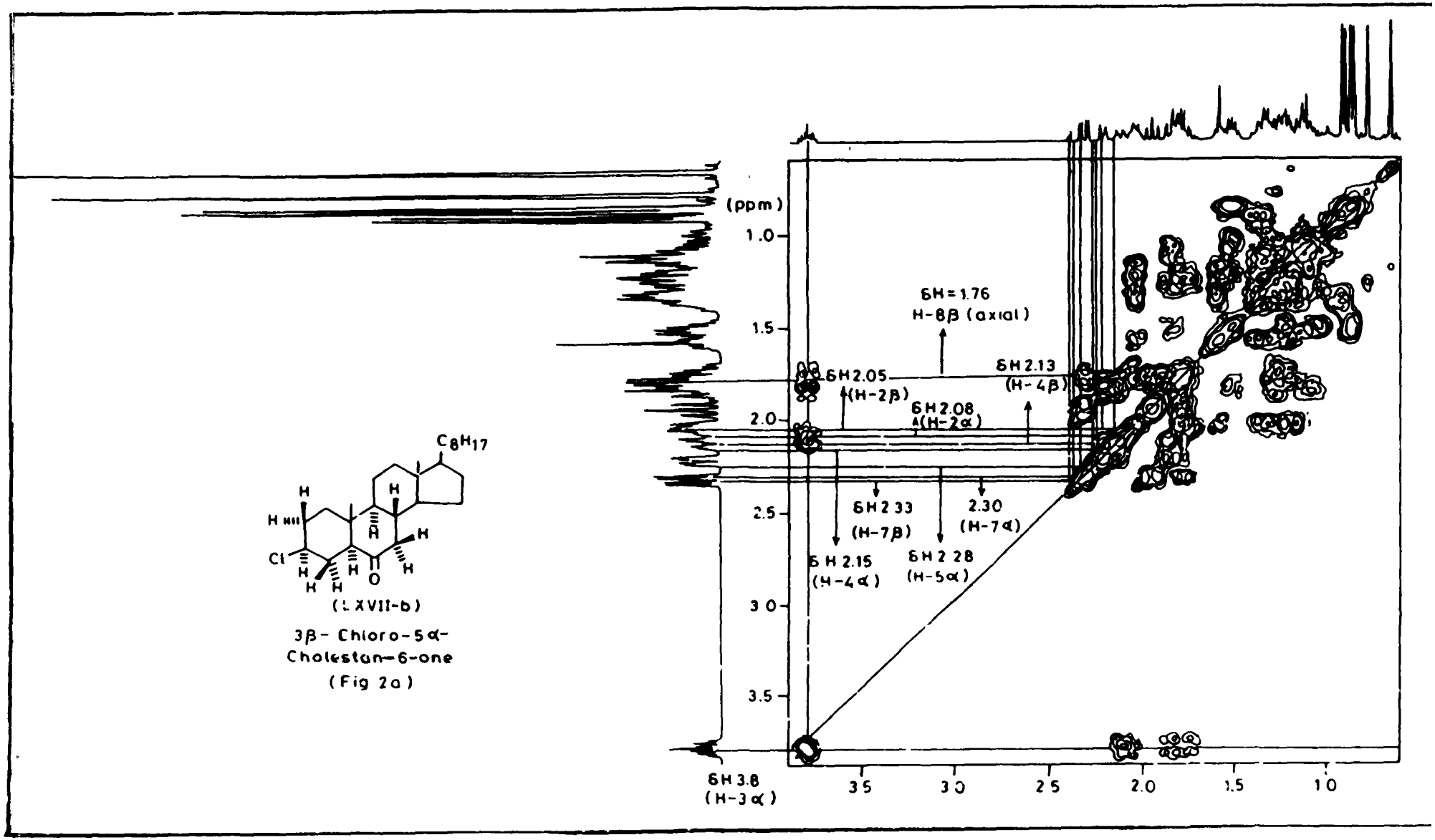
3β-Chloro-6-nitrocholest-5-ene (I) in ethanol is treated with hydrazine hydrate-water in the presence of Raney nickel as per procedure of Forst and Moore[26] at room temperature. After usual work up and column chromatography over silica gel provided crystalline product, m.p. 129°.



(I) **(LXVII-b)**

Compound m.p. 129° as 3β-chloro-5α-cholestan-6-one(LXVII-b) :

The compound (LXVII - b), m.p. 129° (reported, m.p. 128-129°)[29] had analysis $C_{27}H_{45}OCl$ (m/z 420/422, M^{+}) (positive Beilstein test). The I.R. spectrum exhibited bands at 1710 (C=O) and 750 cm^{-1} (C-Cl). The close study of ^{1}H-NMR and ^{13}C-NMR cosy spectra (Fig. 2-a,b) identify coupling protons and correletion with ^{13}carbon.

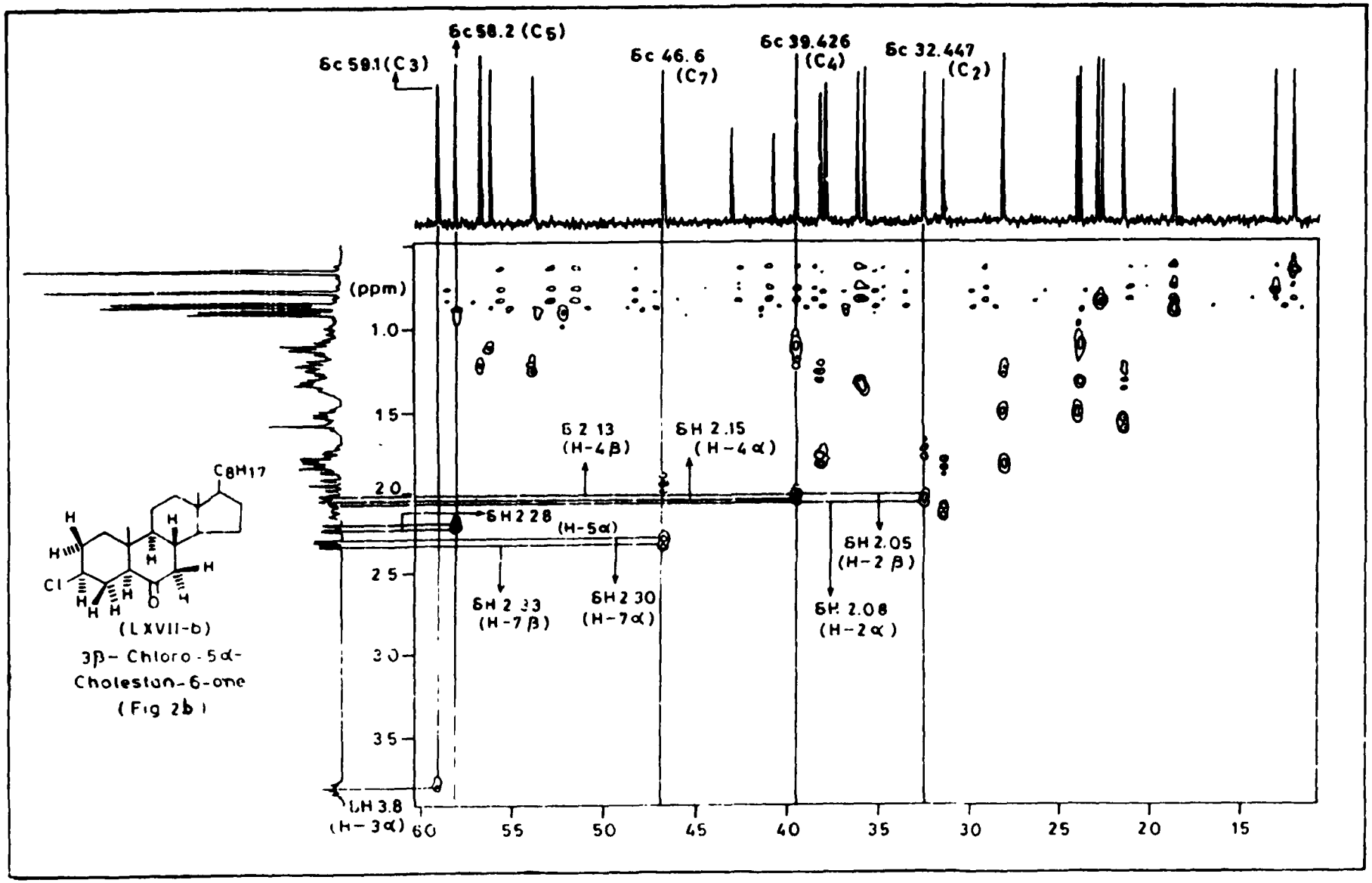

(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
δH=1.76
H-8β (axial)
δH 2.05
(H-2β)
δH 2.13
(H-4β)
δH 2.08
(H-2α)
δH 2.33
(H-7β)
2.30
(H-7α)
δH 2.15
(H-4α)
δH 2.28
(H-5α)
δH 3.8
(H-3α)
3.5
3.0
2.5
2.0
1.5
1.0
C8H17
H
H
H
Cl
O
(LXVII-b)
3β- Chloro-5α-
Cholestan-6-one
(Fig 2a)

^{1}H-^{1}H-NMR homonuclear cosy spectrum of 3β-chloro-5α-cholestan-6-one (LXVII-b) (Fig. 2a) :

The ^{1}H-^{1}H-NMR homonuclear cosy spectrum of (LXVII-b) (Fig. 2-a) gave a multiplet at δ 3.8 as contour on the diagonal and known as diagonal peak multiplet. We find on either side of the diagonal cross peak multiplets at δ 2.13 (H-4β), δ 2.15 (H-4α), δ 2.05 (H-2β) and δ 2.08 (H-2α) coupling with (H-3α). A double doublets at δ 2.33 with Jae=4.5 Hz and Jgem= 13 Hz was assigned to H-7β. This proton being equatorial is coupled by H-8β (δ 1.76, axial) and H-7α (δ 2.30, axial) which is correlated by ^{1}H-NMR cosy spectrum. A double doublet at δ 2.28 (Jae 4.5 Hz and Jaa 13 Hz) for one proton is assigned to (H-5α). This proton being coupled by H-4α at δ 2.15 and H-4β at δ 2.13.

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β - chloro-5α-cholestan-6-one (LXVII-b) (Fig. 2b):

Chemical shifts (as correlated in ^{1}H-NMR homonuclear cosy spectrum) are also easily correlated with ^{1}H-^{13}C-NMR heteronuclear cosy spectrum. δ 3.8 (H-3α) is correlated with δc 59.1 (C3), δ 2.28 (H-5α) to δc 58.2 (C5), δ

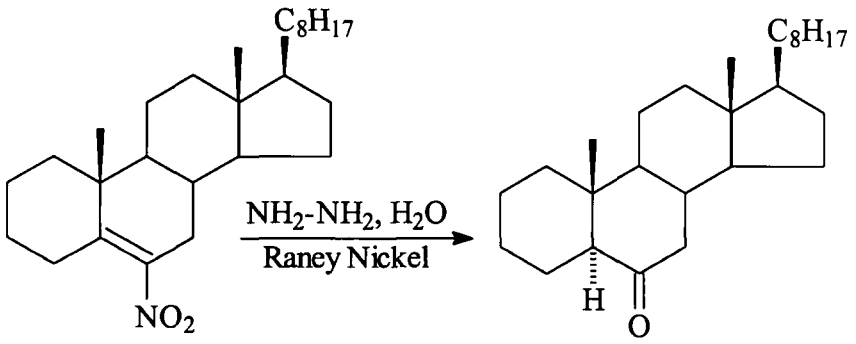

δc 58.2 (C5)
δc 59.1 (C3)
δc 46.6 (C7)
δc 39.426 (C4)
δc 32.447 (C2)
(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
δ 2.13 (H-4β)
δH 2.15 (H-4α)
δH 2.28 (H-5α)
δH 2.05 (H-2β)
δH 2.33 (H-7β)
δH 2.30 (H-7α)
δH 2.08 (H-2α)
δH 3.8 (H-3α)
60
55
50
45
40
35
30
25
20
15
C8H17
H
H
H
H
Cl
H
H
H
H
O
H
(LXVII-b)
3β- Chloro-5α-
Cholestan-6-one
(Fig 2b)

2.33 and δ 2.30 to δc 46.632 (C7) and 2.05 (H-2β) and δ 2.08 (H-2α) are finally correlated to δc 32.447 (C2).

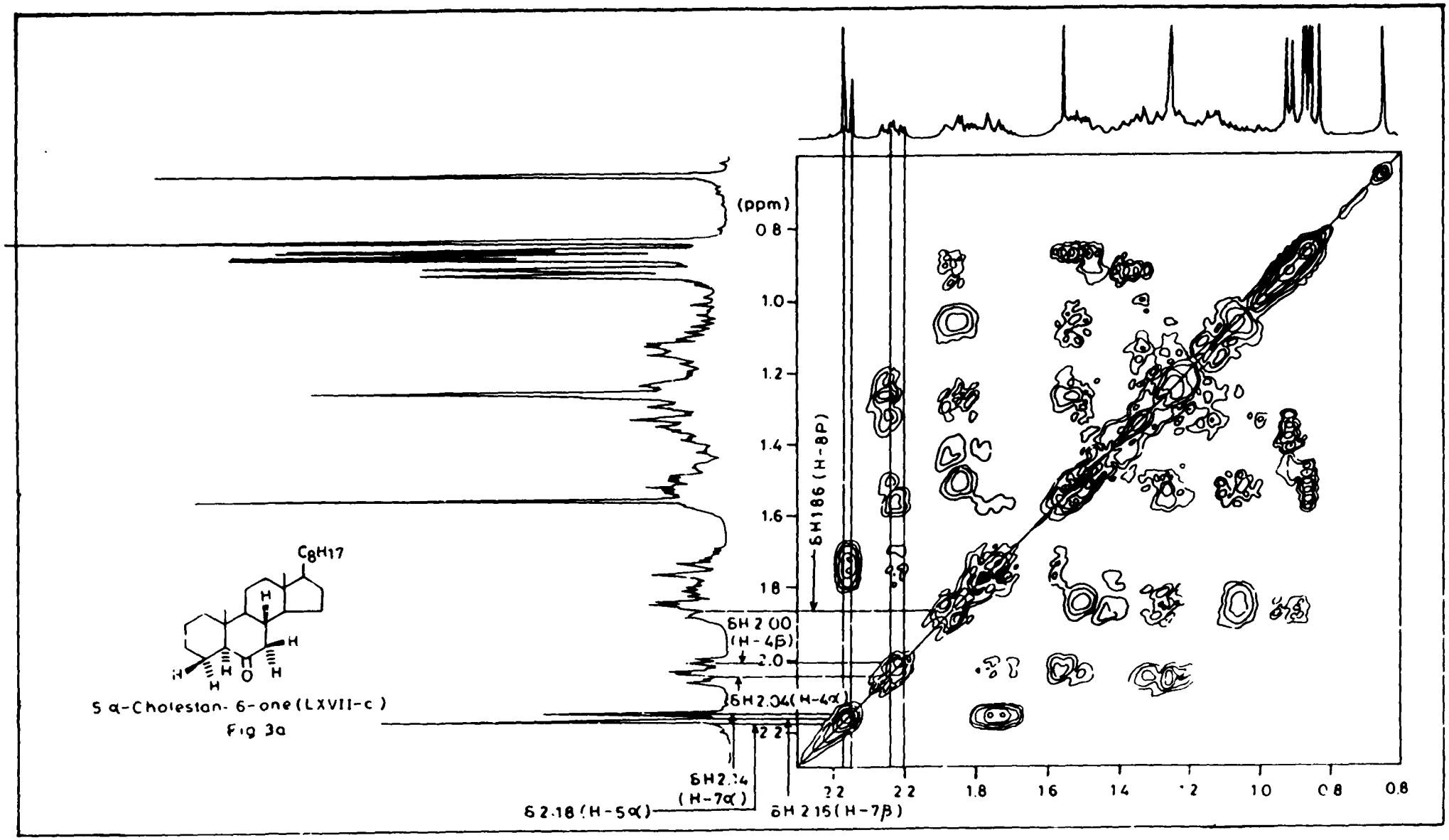
The mass spectrum of 3β-chloro-5α-cholestan-6-one (LXVII-b)gave molecular ion at m/z 420/422 ($M^{+\cdot}$). Other significant fragment ion peaks are at m/z 405/407, ($M^{+\cdot}$ -CH_3), m/z 384 ($M^{+\cdot}$ -HCl), m/z 366 (m/z 384 –H_2O), m/z 307/309 ($M^{+\cdot}$ -C_8H_{17}), m/z 356 (m/z 384 –CO) and ions of lower mass.

Hydrazine-Hydrate Reduction of 6-nitrocholest-5-ene (XXIX) catalysed by Raney Nickel :

6-Nitrocholest-5-ene (XXIX) on reduction with hydrazine hydrate (Raney Nickel as catalyst) following the same procedure provided 5α-cholestan-6-one (LXVII-c), m.p. 97 - 99° (reported m.p.[29a] 98-100°).



(XXIX) **(LXVII-c)**



5 α-Cholestan-6-one (LXVII-c)

Fig 3a

Compound, m.p. 97-99° as 5α-cholestan-6-one (LXVII-c) :

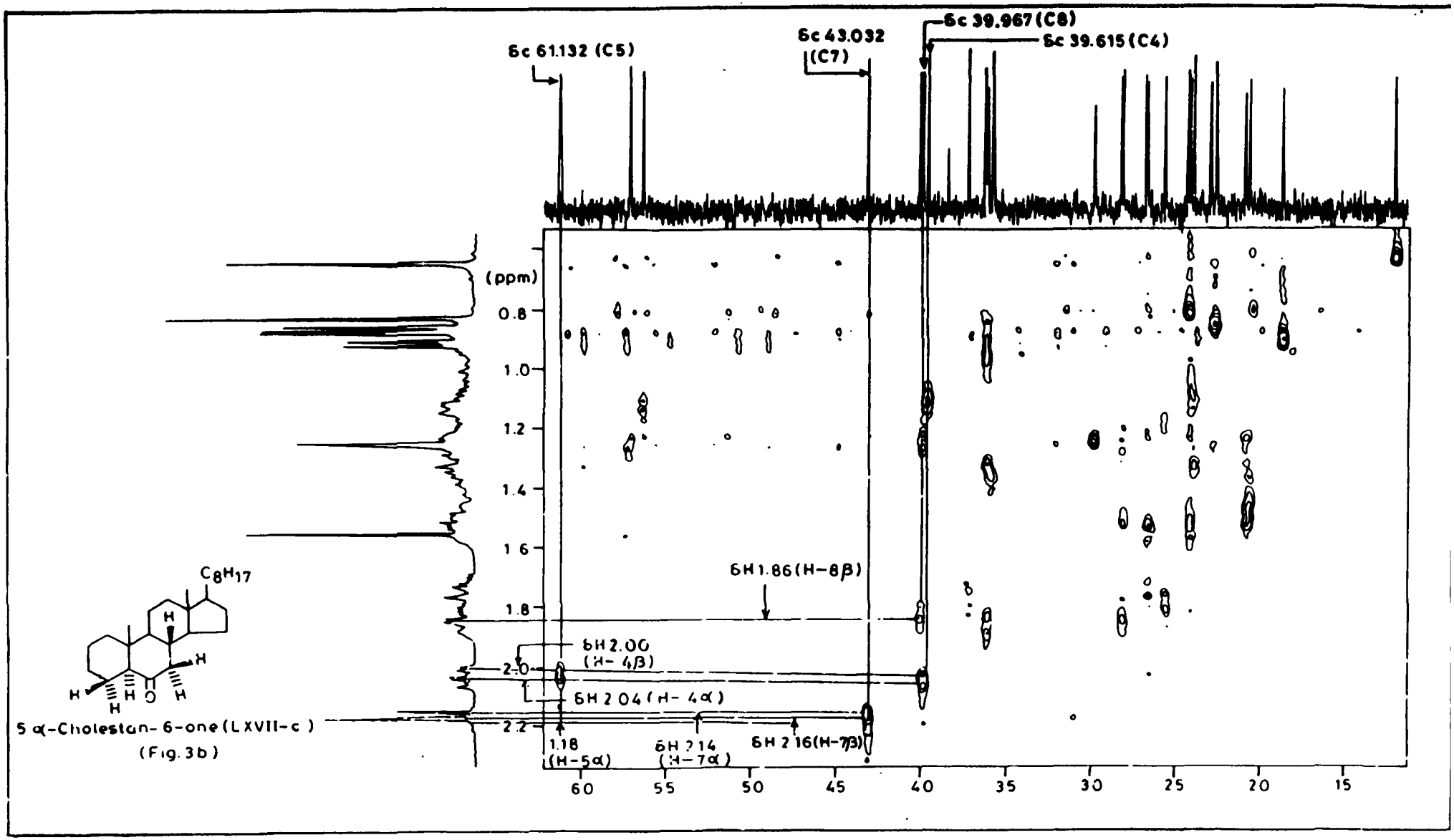
The compound, m.p. 97-99° (reported, m.p.[29a] 98-100°) was analysed for $C_{27}H_{46}O$. In its IR spectrum, a very strong band at 1715 cm^{-1} was found for carbonyl group.

^1H-^1H-NMR homonuclear cosy spectrum of 5α-cholestan-6-one (LXVII-c) (fig. 3a) :

^1H-^1H-NMR homonuclear cosy spectrum of 5α-cholestan-6-one (LXVII-c) (Fig. 3a) explains that H-5α at δ 2.18 as double doublet (Jaa = 13.5 Hz, Jae = 4.5 Hz, axial)[29b] was coupled with H-4α (δ 2.04) and H-4β (δ 2.00). H-7β (δ 2.16) appeared as double doublet (Jea=4.5 Hz and Jgem=13.0 Hz) was coupled with H-7α (δ 2.14) and H-8β (δ 1.86) similarly H-7α (δ 2.14) was coupled with H-7β (δ 2.16) and H-8β (δ 1.86).

^1H-^{13}C-NMR heteronuclear cosy spectrum of 5α-cholestan-6-one (LXVII-c) (Fig. 3b) :

^1H-^{13}C-NMR cosy spectrum of 5α-cholestan-6-one (LXVII-c)(Fig. 3b) correlates H-4α and H-4β (δ 2.04, 2.00) to δ_C 26.656, H-5α (δ 2.18) to δ_C

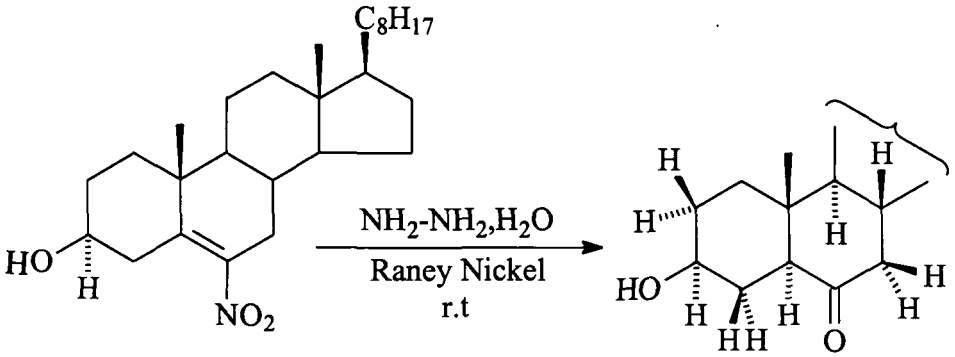

δc 61.132 (C5)
δc 43.032 (C7)
δc 39.967 (C8)
δc 39.615 (C4)
(ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
δH 1.86 (H-8β)
δH 2.00 (H-4β)
δH 2.04 (H-4α)
1.18 (H-5α)
δH 2.14 (H-7α)
δH 2.16 (H-7β)
60
55
50
45
40
35
30
25
20
15
C8H17
H
H
H
H
H
O
5 α-Cholestan-6-one (LXVII-c)
(Fig. 3b)

61.132 (C5) H-7β and H-7α (δ 2.17, 2.15) to δ_C 43.032 and H-8β (δ 1.86) to 39.976 (C8).

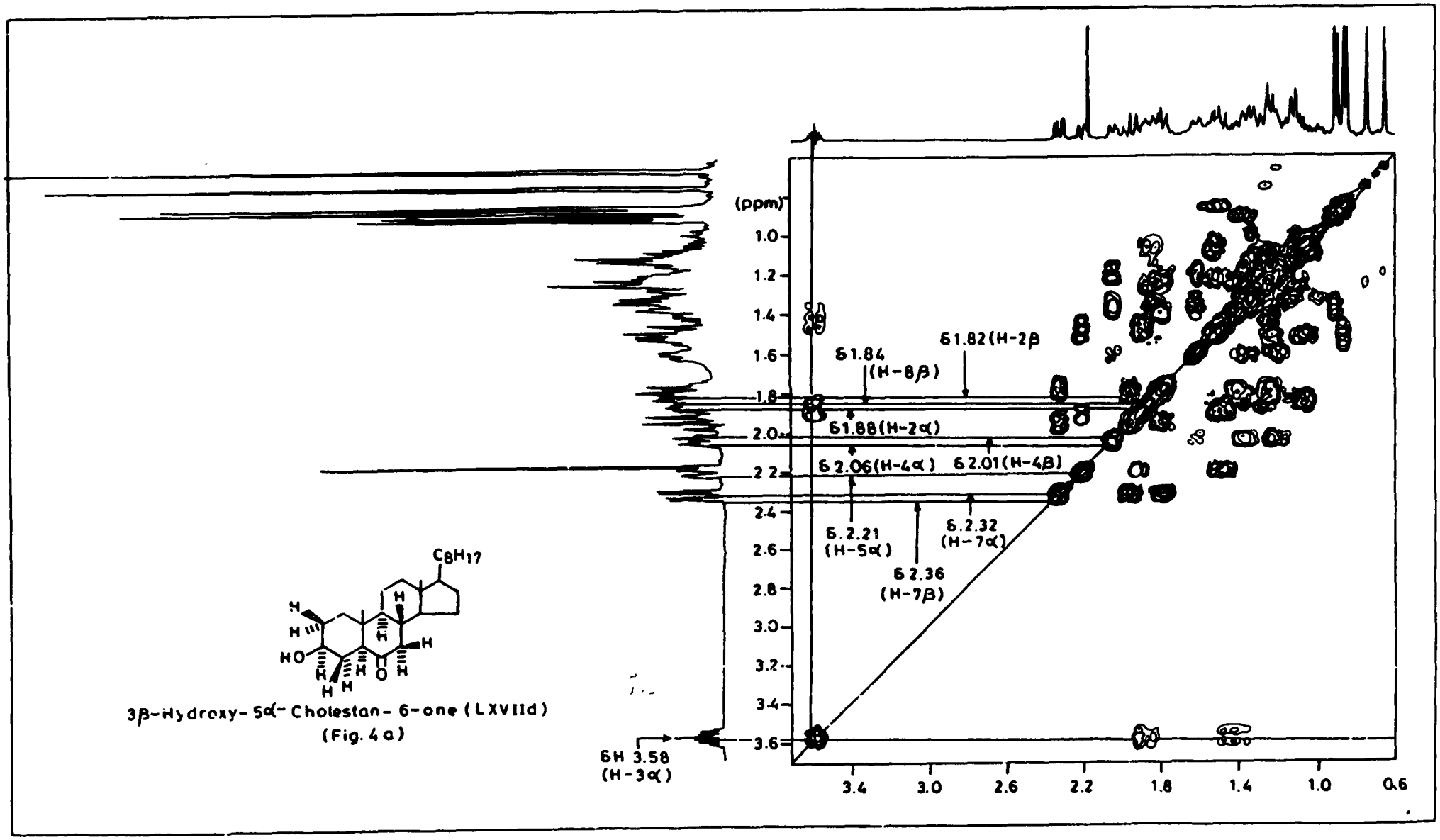
The mass spectrum of 5α-cholestan-6-one (LXVII-c) gave molecular ion peak at m/z 386($M^{+\bullet}$) followed by peaks at m/z 273 ($M^{+\bullet}$ -C_8H_{17}), m/z 368($M^{+\bullet}$ -H_20), m/z 358 ($M^{+\bullet}$ -CO) and lower mass peaks.

Hydrazine-Hydrate Reduction of 3β-hydroxy-6-nitrocholest-5-ene (LXIX) catalysed by Raney Nickel :

3β-Hydroxy-6-nitrocholest-5-ene (LXIX) on hydrazine hydrate reduction (catalysed by Raney Nickel) under identical reaction conditions afforded 3β-hydroxy-5α-cholestan-6-one (LXVII-d) purified by silica gel column chromatography, m.p. 165°C.



(LXIX) **(LXVIII-d)**



3β-Hydroxy-5α-Cholestan-6-one (LXVIId)

(Fig. 4a)

Compound, m.p. 165° as 3β-hydroxy-5α-cholestan-6-one(LXVII-d) :

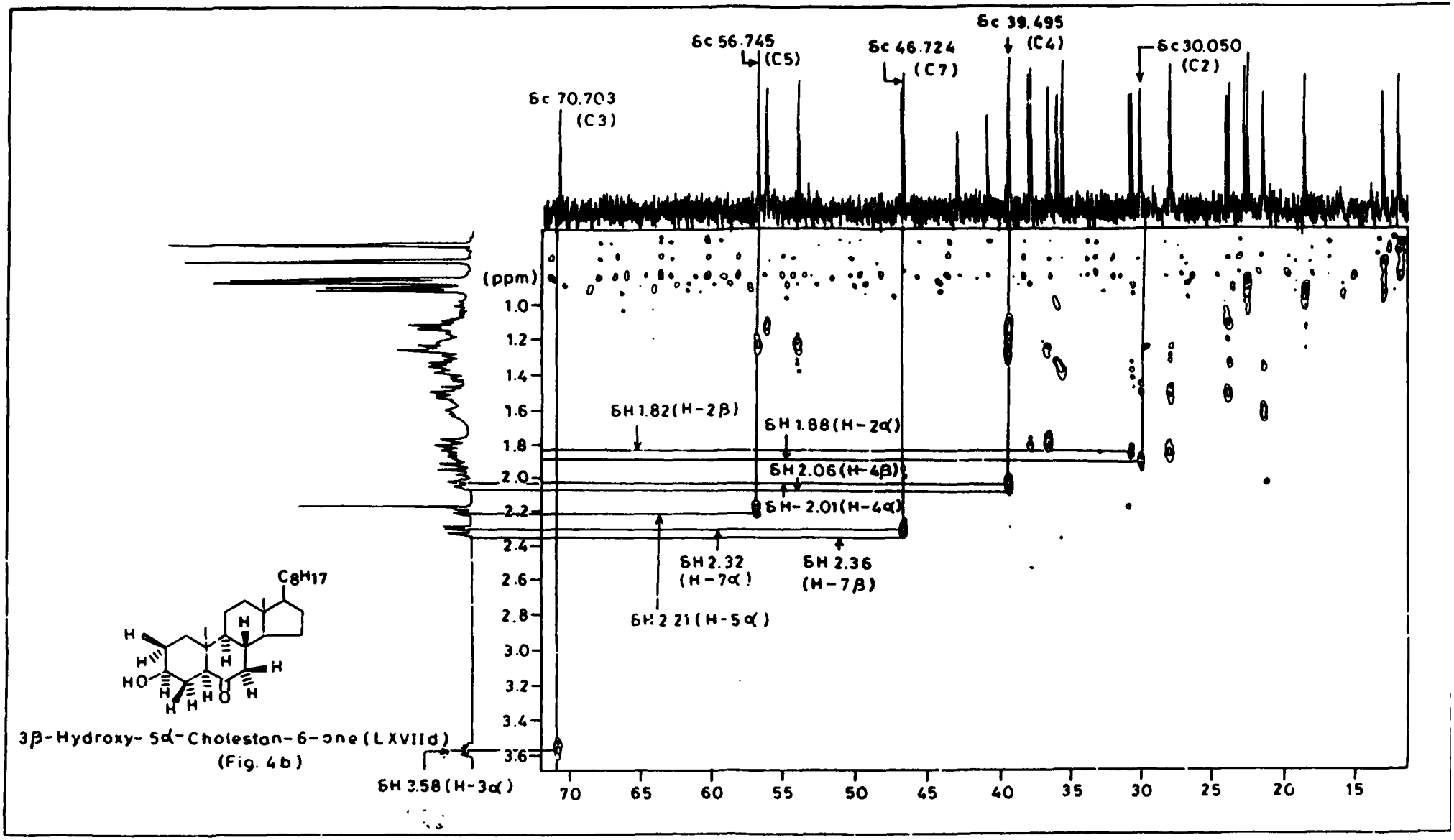
The compound (LXVII-d), m.p. 165° was analysed for $C_{27}H_{46}O_2$ (m/z 402, M+). In its IR spectrum, a broad band at 3400 cm^{-1} for hydroxy group, a band at 1715 cm^{-1} for carbonyl group were observed.

^{1}H-^{1}H-NMR homonuclear cosy spectrum of 3β-hydroxy-5α-cholestan-6-one (LXVII-d) (Fig. 4a) :

^{1}H-^{1}H-NMR cosy spectrum explains that H-3α which gives peak at δ 3.58 (mc, W½ = 18 Hz)[29b] was coupled by H-4α (δ 2.06), H-4β (δ 2.01), H-2α (δ 1.88) and H-2β (δ 1.82). H-5α at δ 2.21 is coupled by H-4α (δ 2.06) and H-4β (δ 2.01). H-7β at δ 2.36 is coupled by H-7α (δ 2.32) and H-8β (δ 1.84). H-7α at δ 2.32 is coupled by H-7β (δ 2.36) and H-8β (δ 1.84).

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3β-hydroxy-5α-cholestan-6-one (LXVII-b) (Fig. 4b) :

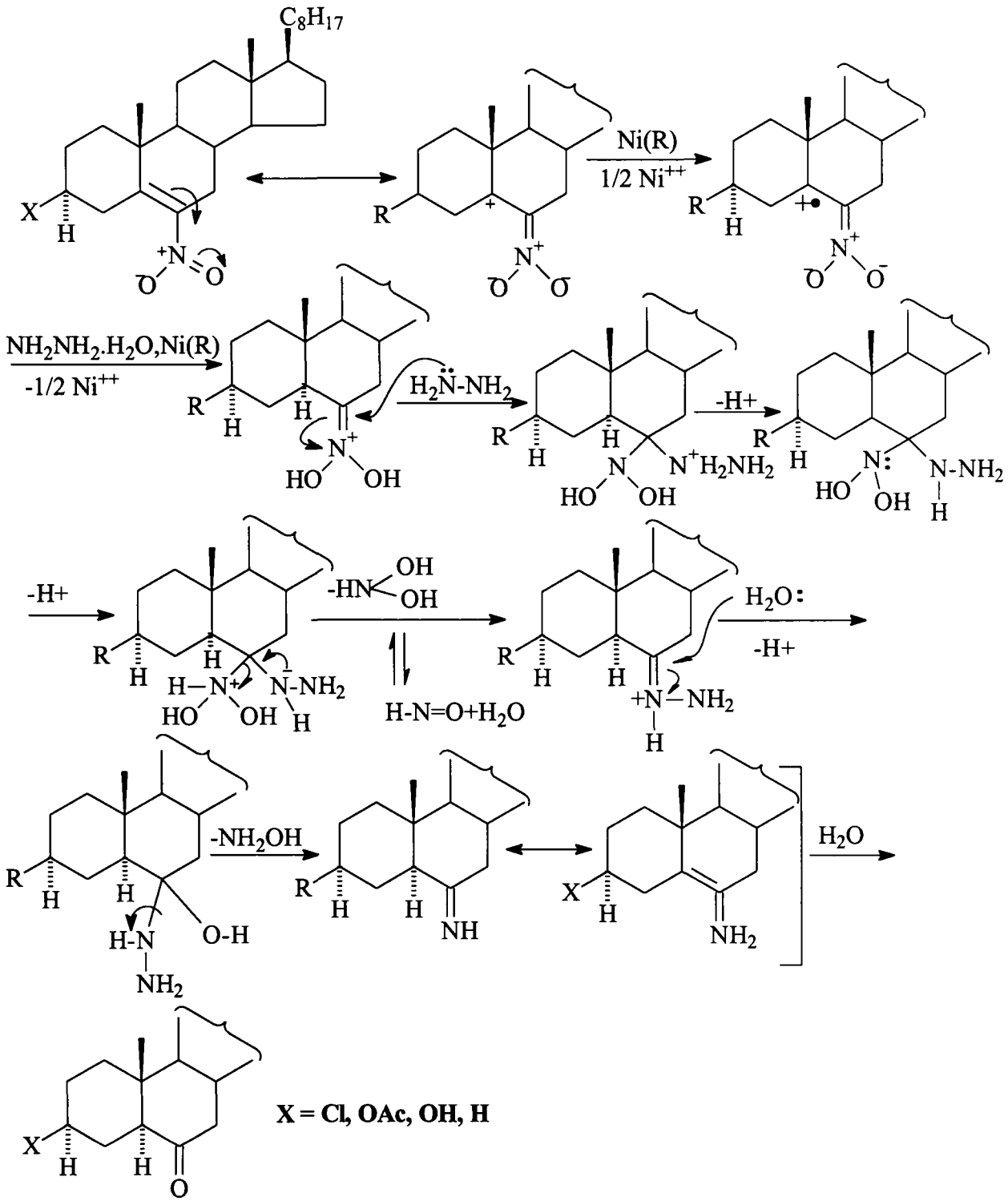
^{1}H-^{13}C-NMR cosy spectrum of 3β-hydroxy-5α-cholestan-6-one (LXVII-b) correlates the H-2α (δ 1.88), H-2β (δ 1.82) to δ_C 30.050 (C2), H-3α (δ 3.58) to δ_C 70.703 (C3), H-4α (δ 2.06) and H-4β (δ 2.01) at δ_C 39.495


δc 56.745 (C5)
δc 46.724 (C7)
δc 39.495 (C4)
δc 30.050 (C2)
δc 70.703 (C3)
(ppm)
δH 1.82 (H-2β)
δH 1.88 (H-2α)
δH 2.06 (H-4β)
δH- 2.01 (H-4α)
δH 2.32 (H-7α)
δH 2.36 (H-7β)
δH 2.21 (H-5α)
δH 3.58 (H-3α)
C8H17
3β-Hydroxy-5α-Cholestan-6-one (LXVIId)
(Fig. 4b)

(C4), H-5α at δ 2.21 to δ_C 56.745, H-7β at δ 2.36 and H-7α at δ 2.32 are correlated to δ_C 46.724 (C7). The H-8β proton is correlated to δ_C 37.909 (C8).

The mass spectrum of 3β-hydroxy-5α-cholestan-6-one (LXVII-b) gave molecular ion peak at m/z 402 ($M^{+\cdot}$) followed by peaks due to the loss of methyl group m/z 387 ($M^{+\cdot}$ -CH_3) and water molecule ($M^{+\cdot}$ -H_2O) and fragment ion peaks of lower mass.

Since the total transformation of vinyl nitro steroids to ketones by hydrazine-hydrate-Ni(R) occurred at room temperature with 90% yields confirmed that hydrazine-hydrate-Ni(R) is better reducing agent ($Ni \rightarrow \frac{1}{2} Ni^{++}$, $+ 0.263\ V + N_2H5^+ + 3H^+ + 2e \rightarrow 1.27\ V$)[24], *than Zn-AcOH combination.* A tentative hydrazine-hydrate-Nickel(R) reduction is proposed as follows.


C8H17
X
H
+N
O
O
R
+
N+
O
O
Ni(R)
1/2 Ni++
R
H
+•
N+
O
O
NH2NH2.H2O,Ni(R)
-1/2 Ni++
R
H
H
N+
HO
OH
H2N-NH2
R
H
H
N
N+H2NH2
HO
OH
-H+
R
H
N:
N-NH2
HO
OH
H
-H+
R
H
H
H—N+
N-NH2
HO
OH
H
-HN
OH
OH
H-N=O+H2O
H2O:
-H+
R
H
H
+N—NH2
H
-NH2OH
R
H
H
H-N
O-H
NH2
R
H
H
NH
X
H
NH2
H2O
X
H
H
O
X = Cl, OAc, OH, H

EXPERIMENTAL

All melting points are uncorrected. Infrared spectra (IR) were measured in KBr with Perkin-Elmer 237 and Unichem SP300 spectrophotometeres. The NMR spectra were run in $CDCl_3$ on Varian A60 instrument with TMS as internal standard. Mass spectra were run on JEOL JMS D30 mass spectrophotometer at the source temperature of 120°C. Thin layer chromatographic plates were coated with silica gel G and sprayed with a 20% aqueous solution of perchloric acid. Light petroleum refers to a fraction of b.p. 60-80°. NMR values were given in ppm (s, singlet; d, doublet; t, triplet; br, broad; m, multiplet centred at). IR values are given in cm^{-1} (s, strong; m, medium; w, weak; br, broad).

3β-Chlorocholest-5-ene (LXX)

Freshly purified thionyl chloride (37 ml) was added gradually to cholesterol (50 g) at room temperature. A vigorous reaction ensued with the evolution of gaseous products. When the reaction slackened, the mixture was gently heated at a temperature of 50-60° on a water bath for 1 hour and then poured on to crushed ice with stirring. The yellow solid thus obtained was filtered under suction and washed several times with ice-cold water and air-

dried. Recrystallization from acetone gave 3β-chloro-cholest-5-ene (LXX) (47.5 g), m.p. 95-96° (reported[30], m.p. 96-97°). It gave a positive Beilstein test and a yellow colour with tetranitromethane in chloroform.

3β-Acetoxycholest-5-ene (LXXI)

A mixture of cholesterol (LX) (100.0 g), pyridine (150 ml) and freshly distilled acetic anhydride (100 ml) was heated on a water bath for 2 hours. A light brown solution was obtained which, after allowing to cool at room temperature was poured on to crushed ice with stirring. 3β-Acetoxy-cholest-5-ene (LXXI) was obtained as a white precipitate, which was filtered under suction, washed with water and air-dried. The crude product was recrystallized from acetone as needles, m.p. 114-115° (reported[22], m.p. 116°).

Cholest-5-ene (LXXII)

3β-Chlorocholest-5-ene (LXX) (10.0 g) was dissolved in warm amyl alcohol (230 ml) and sodium metal (20.0 g) was added to the solution with continuous stirring over a period of 8 hours. During this period of addition of sodium, the reaction mixture was warmed occasionally so as to facilitate the dissolving of sodium metal. When all the sodium metal was dissolved, the

reaction mixture was poured into water, acidified with hydrochloric acid and then allowed to stand over night. A white crystalline solid was obtained which was filtered under suction and washed thoroughly with water and air-dried. The crude material was recrystallized from acetone to provide (LXXII) as cubes (7.5 g), m.p. 94° (reported[31], m.p. 89.5-91.2°).

3β-Chloro-6-nitrocholest-5-ene (I)

To a well stirred mixture of 3β-chlorocholest-5-ene (LXX) (12 g), glacial acetic acid (80 ml) and nitric acid (25 ml, d, 1.52) at temperature below 20°, was added sodium nitrite (3.0 g) gradually. After the complete addition of sodium nitrite, the mixture was further stirred for 1 hour. Ice cold water (200 ml) was added and the yellowish solid thus separated was filtered and air-dried. The desired product (I) was recrystallized from ethanol as needles (9.0 g), m.p. 150-152° (reported[32], m.p. 149°).

3β-Acetoxy-6-nitrocholest-5-ene (IX)

To a mixture of 3β-acetoxy cholest-5-ene (LXXI) (10.0 g) and nitric acid (250 ml, d, 1.42), sodium nitrite (10.0 g) was gradually added over a period of 1 hour with continuous stirring. Slight external cooling was also

applied during the course of reaction and stirring was continued for about 2 hours when a yellow spongy mass separated on the surface of the mixture. The mixture was then diluted with cold water (200 ml) when a green coloured solution was obtained. The whole mass was extracted with ether and the ethereal solution was successively washed with water, sodium bicarbonate solution (5%) (until the washings attained pink colour) and water and dried over anhydrous sodium sulphate. Removal of the solvent afforded (IX) as an oil which was crystallized from methanol (with a trace of acetone) (7.2 g), m.p. 104° (reported[33], m.p. 103-104°).

3β-Hydroxy-6-nitrocholest-5-ene (LXIX)

To a well stirred solution of 3β-hydroxycholest-5-ene (LX) (28.0 g) in glacial acetic acid (300 ml) and nitric acid (12.5 ml, d, 1.52) at temperature below 20°, was added sodium nitrite (1.5 g) gradually. After the complete addition of sodium nitrite, the mixture was further stirred for 1 hour. Ice cold water (100 ml) was added and the yellowish solid thus separated was filtered and air-dried. The desired product (LXIX) was recrystallized from ethanol as needles (4.5 g), m.p. 130° (reported[34], m.p. 129-131°).

6-Nitrocholest-5-ene (XXIX)

A suspension of finely powdered cholest-5-ene (LXXII) (6.0 g) in glacial acetic acid (50 ml) was vigorously stirred at room temperature and treated slowly with nitric acid (15 ml, d, 1.5) followed by the addition of sodium nitrite (3 g) over a period of 1 hour. The reaction mixture was poured into cold water and the yellow product thus obtained was extracted with ether. The ethereal solution was washed successively with water, sodium bicarbonate solution (5%) (until the washings were pink) and again with water. Removal of the solvent, after drying over anhydrous sodium sulphate, provided (XXIX) as an oil which was crystallized from ethanol in leaflets (4.00 g), m.p. 119-120° (reported[35], m.p. 120-121°).

Reaction of 3β-Chloro-6-nitrocholest-5-ene (I) with Zn-AcOH[23]:

3β-Chloro-5α-cholestan-6-one (LXVII-b)

To a solution of 3β-chloro-6-nitrocholest-5-ene (I) (6.0 g) in hot glacial acetic acid (120 ml), zinc dust (12.0 g) was added gradually in small portions with shaking. The suspension was heated under reflux for 4 hours and water (12 ml) was added at regular intervals during the course of heating. The hot solution was filtered to remove zinc powder and cooled to room temperature

followed by dilution with large excess of ice-cold water. The organic matter was extracted with ether and the ethereal solution was washed with sodium bicarbonate solution (10%) and water, and dried over anhydrous sodium sulphate. Evaporation of the solvent furnished (LXVII - b) as an oil which was crystallized from methanol (3.8 g), m.p. 127-129° (reported[28], m.p. 129°).

Reaction of 3β-chloro-6-nitrocholest-5-ene (I) with hydrazine hydrate-Raney nickel : 3β-Chloro-5α-cholestan-6-one(LXVII-b)

3β-Chloro-6-nitrocholest-5-ene (I) (3 g) in ethanol (100 ml) was treated with hydrazine hydrate (5 ml, 100%) in the presence of Raney nickel (0.75 g) at ambient temperature. After the completion of the reaction, the reaction mixture was filtered. The filtrate was diluted with water and extracted with ether. The ethereal layer was washed with water, sodium hydrogen carbonate solution (5%) and water. The extract was dried over anhydrous sodium sulphate. Evaporation of the solvent provided a residue (2.7 g) which was chromatographed over a column of silica gel (30 g).

3β-Chloro-5α-cholestan-6-one (LXVII -b) :

Elution : pet. ether : ether (20:1), solvent of crystallization : methanol, Yield : (2.1 g), m.p. 129° (reported[28], m.p. 129-130°).

Analysis found : C, 76.88; H, 10.56

Requires ($C_{27}H_{45}OCl$) : C, 77.14; H, 10.71%

IR : ν max 1710 (C-O), 750 cm^{-1} (C-Cl).

^{1}H-NMR ($CDCl_3$) : δ3.8 (mc, 1H, W½ =18 Hz, axial, H-3α), 2.33 (dd, 1H, Jae 4.5 Hz, gem 13 Hz, H-7β), 2.30 (t, 1H, H-7α), 2.28(dd, 1H, Jea, 4.5 Hz, Jaa 13 Hz, H-5α)[29b] 1.10 (C10-CH_3) and 0.65 (Cl3-CH_3), 0.90 and 0.86 (side chain methyl protons).

^{13}C-NMR ($CDCl_3$) : δc 32.447(C2), 59.1(C3), 39.426(C4), 58.057(C5), 209.903(C6), 46.632(C7), 35.671(C8).

Mass : m/z 420/422 ($M^{+\bullet}$), m/z 405/407 ($M^{+\bullet}$ -CH_3), m/z 384 ($M^{+\bullet}$ -HCl), m/z 366 (m/z 384 – H_2O), m/z 356 (m/z 384 – CO), m/z 307/309 ($M^{+\bullet}$ -C_8H_{17}) and fragment ion peak of lower mass.

Reaction of 3β-acetoxy-6-nitro cholest-5-ene (IX) with Zn AcOH : 3β-Acetoxy-5α-cholestan-6-one (LXVII - a)

3β-Acetoxy-6-nitrocholest-5-ene (IX) (10.0 g) was dissolved in glacial acetic acid (200 ml) and zinc dust (20.0 g) was added in small portions with shaking. The suspension was heated under reflux for about 4 hours and water (20 ml) was added at regular intervals during the course of heating. The hot solution was filtered, cooled to room temperature and diluted with a large excess of ice-cold water. The precipitate thus obtained was taken in ether and the ethereal solution was washed with water, sodium bicarbonate solution (10%) and water and then dried over anhydrous sodium sulphate. Evaporation of the solvent gave (LXVII-a) which was crystallized from methanol (6.2 g), m.p. 128-129° (reported[29], m.p. 127-128°).

Reaction of 3β-acetoxy-6-nitrocholest-5-ene (IX) with hydrazine hydrate-Raney nickel : 3β-Acetoxy-5α-cholestan-6-one (LXVII - a)

To a solution of 3β-acetoxy-6-nitrocholest-5-ene (IX) (3 g) in ethanol (100 ml) was added hydrazine hydrate (5 ml, 100%). Raney nickel (0.75 g) was added in small portions and the temperature of the reaction mixture was

maintained below 30°. It was kept at room temperature for 2-3 hours. On completion of the reaction (checked by running TLC at frequent interval) the mixture was filtered and the filtrate was diluted with water and extracted with ether. The ethereal solution was washed with sodium hydrogen carbonate solution (5%) and water an dried over anhydrous sodium sulphate. Evaporation of the solvent provided an oily residue which was chromatographed over a column of silica gel (60 g).

3β-Acetoxy-5α-cholestan-6-one (LXVII-a) :

Elution : pet. ether : ether (20:1), solvent of crystallization : methanol, Yield : (2.2 g), m.p. 128-129° (reported[29], m.p. 127-128°).

Analysis found: C, 78.21; H, 10.76

$C_{29}H_{48}O_3$: C, 78.38; H, 10.81%

IR : ν max 1735 (-O-CO-CH_3), 1710 (>C=O), 1210, 1035 cm^{-1} (C-O).

^{1}H-NMR ($CDCl_3$) : δ 4.68 (mc, 1H, W½ = 17 Hz; axial, H-3α)[29b], 2.35 (dd, 1H, Jae = 4.5 Hz, Jgem = 12 Hz H-7β), 2.30 (t, 1H, H-7α), 2.26 (dd, 1H, Jae 4.5 Hz, Jaa 12 Hz H-5α) 2.18 (s,

3H, -O-CO-CH_3), 1.02 (C10 – CH_3), 0.71 (C13 – CH_3), 0.92 and 0.84 (side chain methyl proton)

^{13}C-NMR ($CDCl_3$) : δ_C 72.847(C3), 26.8 (C4), 56.432 (C5), 170.6(C6), 46.6(C7).

Mass : m/z 444($M^{+\bullet}$), m/z 384($M^{+\bullet}$ -CH_3COOH), m/z 366 (m/z 384-H_2O).

3β-hydroxy-5α-cholestan-6-one (LXVII-d)

To a solution of 3β-hydroxy-6-nitrocholest-5-ene (LXIX) (3.0 g) in hot glacial acetic acid (60 ml), zinc dust (6.0 g) was added gradually in small portions with shaking. The suspension was heated under reflux for 4 hours and water (6 ml) was added at regular intervals during the course of heating. The hot solution was filtered to remove zinc dust powder and the filtrate was cooled at room temperature followed by dilution with large excess of ice-cold water. The organic matter was extracted with ether and the ethereal solution was washed with water, sodium biocarbonate solution (10%) and water and dried over anhydrous sodium sulphate. Evaporation of the solvent furnished (LXVII-d) as an oil which was crystallized from methanol (1.85), m.p. 165°C.

Reaction of 3β-hydroxy-6-nitrocholest-5-ene (LXIX) with hydrazine-hydrate-Raney nickel : 3β-Hydroxy-5α-cholestan-6-one (LXVII-d)

To a solution of 3β-hydroxy-6- nitrocholest-5-ene (LXIX) (3 g) in ethanol (100 ml) was added hydrazine hydrate (5 ml, 100%). Raney-nickel (0.75 g) was added in small portions and the temperature of the reaction mixture was maintained below 30°. It was kept at room temperature for 2-3 hours. On completion of the reaction (checked by running TLC at frequent interval) the mixture was filtered and the filtrate was dilution with water and extracted with ether. The ethereal layer was washed with water, sodium hydrogen carbonate solution (5%) and water and dried over anhydrous sodium sulphate. Evaporation of the solvent provided an oily residue which was chromatographed over a column of silica gel (120 g).

3-β-Hydroxy-5α-cholestan-6-one (LXVII-d)

Elution : pet. ether : ether (20:1), solvent of crystallization : methanol, Yield : (2.35 g), m.p. 165°.

Analysis found : C, 80.39; H, 10.98

$C_{27}H_{46}O_2$ requires : C, 80.60; H, 11.44%

IR : ν max 3400 (-OH), 1715 cm^{-1} (C=O)

^{1}H-NMR ($CDCl_3$) : δ3.58 (mc 1H, W½= 18 Hz, H-3α)[24b], 2.36 (dd, 1H, Jae 4.5 Hz, Jgem 13 Hz, H-7β), 2.30 (t, 1H, H-7α), 2.20 (brs, 1H, -OH), 2.18 (dd, 1H, Jae 4.5 Hz, Jaa 12 Hz, H-5α), 1.15 (C10-CH_3), 0.71 (C13-CH_3), 0.91 and 0.86 (side chain methyl protons).

^{13}C-NMR ($CDCl_3$) : δ_C30.050 (C2), 70.58 (C3), 39.495 (C4), 56.745 (C5), 210.882 (C6), 46.724 (C7), 37.909 (C8).

Mass : m/z 402 (M^+), m/z 387 (M^+ -CH_3), m/z 384 (M^+ - H_2O), and fragment ion peaks of lower mass.

5α-Cholestan-6-one (LXVII-c)

6-Nitrocholest-5-ene (XXIX) (3 g) was dissolved in warm glacial acetic acid (60 ml), and water (6 ml) and zinc dust (6.0 g) was gradually added with shaking. The mixture was refluxed for 4 hours. After the completion of the reaction zinc powder was removed by filtration and the filtrate was diluted

with water and was allowed to stand over night at room temperature. The solid crystallized out, which was filtered and recrystallized from methanol (1.90 g) m.p. 97-99° (reported[29a], m.p. 98-100° C).

Reaction of 6-nitrocholest-5-ene (XXIX) with hydrazine hydrate Raney nickel ; 5α-Cholestan-6-one (LXVII-c)

6-Nitrocholest-5-ene (XXIX) (3 g) was treated with hydrazine hydrate (5 ml, 100%), Raney nickel (0.75 g) as in the previous case. Work up of the reaction mixture followed by evaporation of the solvent yield a brown residue which was chromatographed over silica gel (60 g).

5α-Cholestan-6-one (LXVII-c)

Elution : pet. ether : ether (20:1), solvent of crystallization : methanol, Yield : (2.3 g), m.p. 97-99° (reported[3], m.p. 98-100°).

Analysis found : C, 83.71; H, 11.61

$C_{27}H_{46}O$ requires : C, 83.94; H, 11.92%

IR : ν max 1715 cm^{-1} (C=O)

^{1}H-NMR ($CDCl_3$) : δ 2.18 (dd, 1H, Jaa 13.5 Hz, Jae = 4.5 Hz, H-5α)[29], 2.16 (dd, 1H, Jea = 4.5 Hz, Jgem = 13 Hz, H-7β), 2.14 (t, 1H, H-7α), 1.13 (C10-CH_3), 0.7 (C13-CH_3), 0.9 and 0.85 (side chain methyl protons).

^{13}C-NMR($CDCl_3$) : δ_C 39.615 (C4), 61.132 (C5), 215.754 (C6-CO-), 43.032 (C7), 39.967 (C8).

Mass : m/z 386 ($M^{+\bullet}$), m/z 371 ($M^{+\bullet}$ -CH_3), m/z 368 (M+ -H_2O), m/z 358 ($M^{+\bullet}$ -CO), m/z 273 ($M^{+\bullet}$ -C_8H_{17}).

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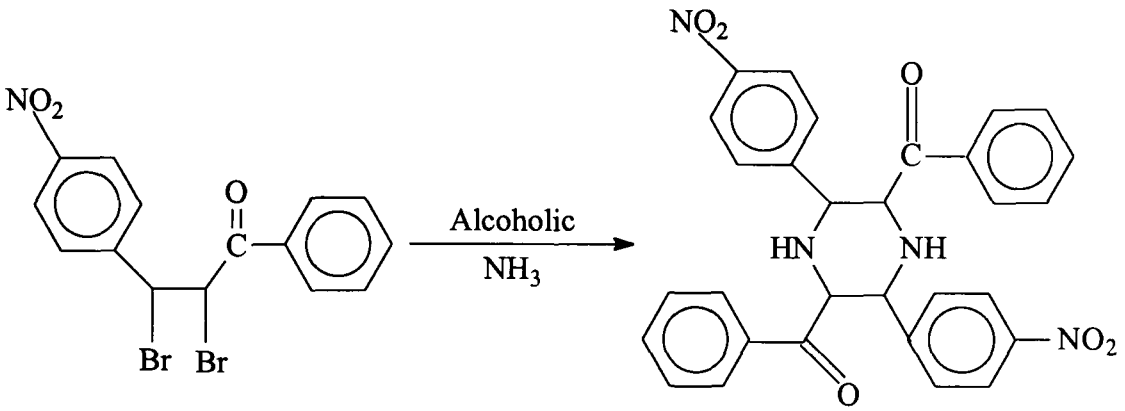
CHAPTER - 3

Reactions of dibromosteroids with Organic bases

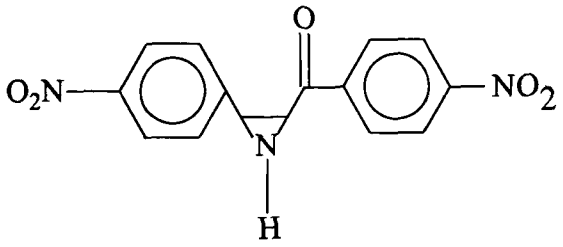
THEORETICAL

N-Containing compounds were known for their extensive utility as potential drugs, and useful intermediates[1,2] in organic synthesis. These compounds play a vital role in the biological systems[3]. The first reported reaction of a bromoderivative of an unsaturated ketone with an amino compound seems to have been the reaction of dibromobenzalacetophenone with ammonia, carried out by Weiland[4] in 1904. Reaction of p-nitrobenzalacetophenone (I) with alcoholic ammonia afforded compound (II).



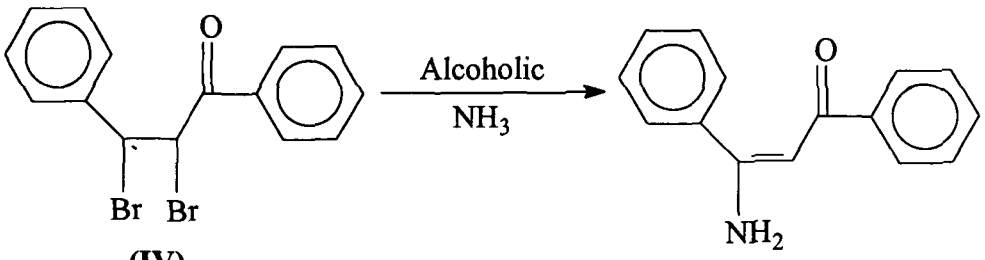
(I) (II)

Later on the structure of this reaction product (II) brought out to be ethylenimine of the type (III), as proven by Cromwell et. al.[5]



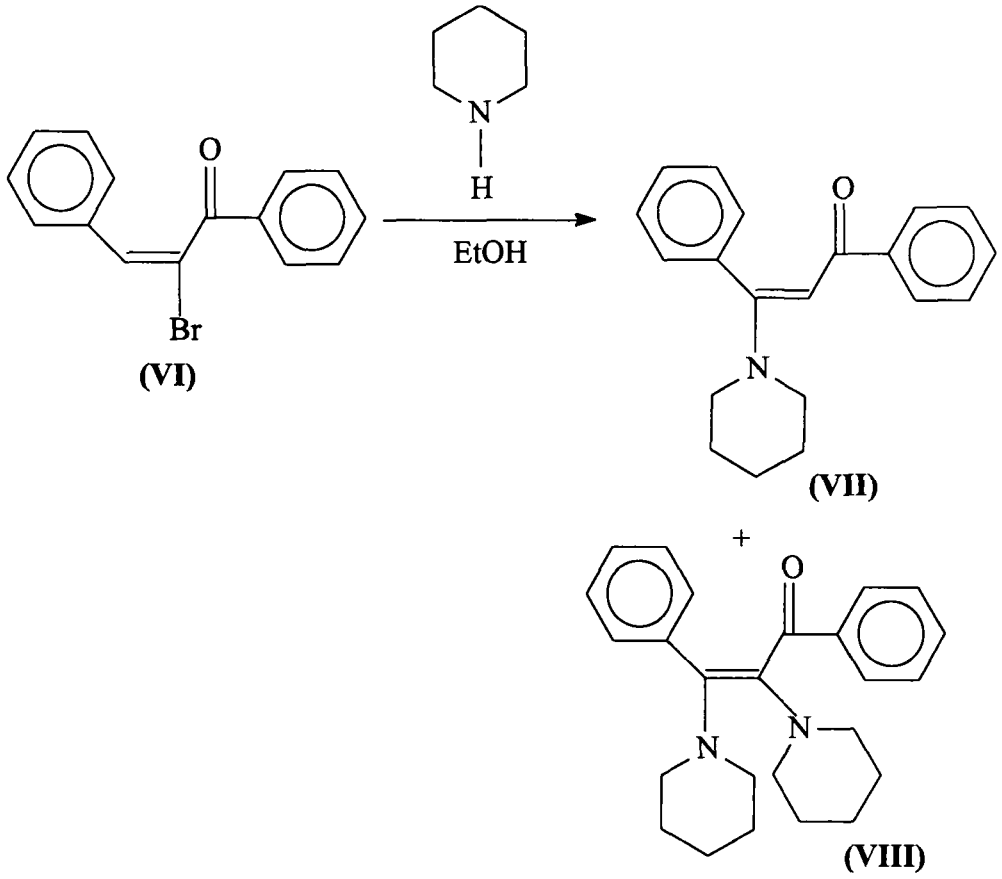
(III)

Ruhemann and Watson[6] reported the reaction of alcoholic ammonia with α,β-dibromobenzalacetophenone (IV) and obtained a new colourless base (V), m.p. 97°C.

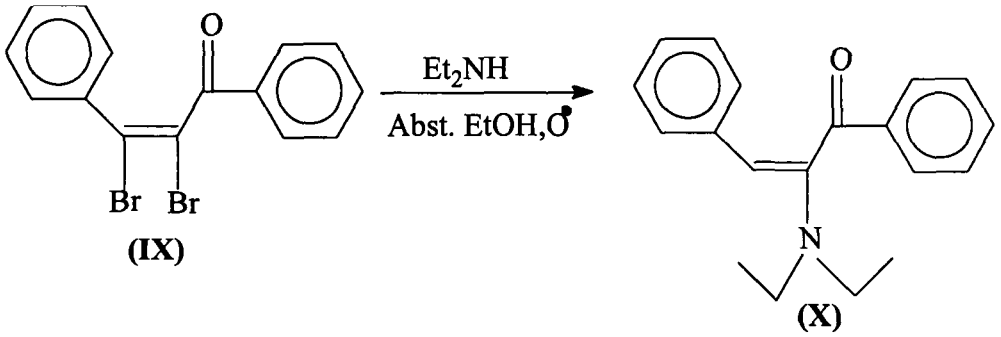


(IV) **(V)**

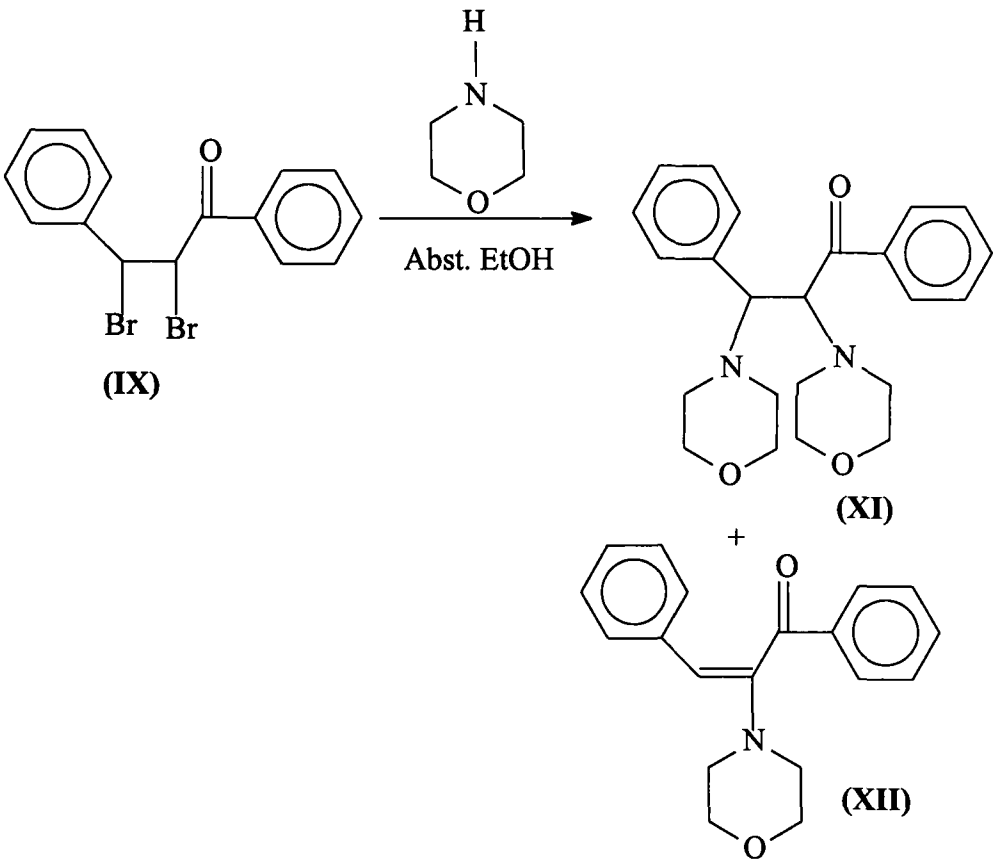
Waston[7] obtained from (VI), β-piperidinobenzalacetophenone (VII) and α,β-dipiperidinobenzalacetophenone (VIII) with piperidine in alcohol.



Cromwell and co-workers reported[8] that dibromobenzylacetophenone (IX) when reacted with excess of diethylamine in alcoholic solution afforded α-N-diethylaminobenzalacetophenone (X) in good yield.



The rapid reaction of benzalacetophenone dibromide (IX) with morpholine had been found to give mostly α,β-dimorpholinobenzalacetophenone (XI) with small amounts of α-morpholinobenzalacetophenone (XII)[9].



(IX)

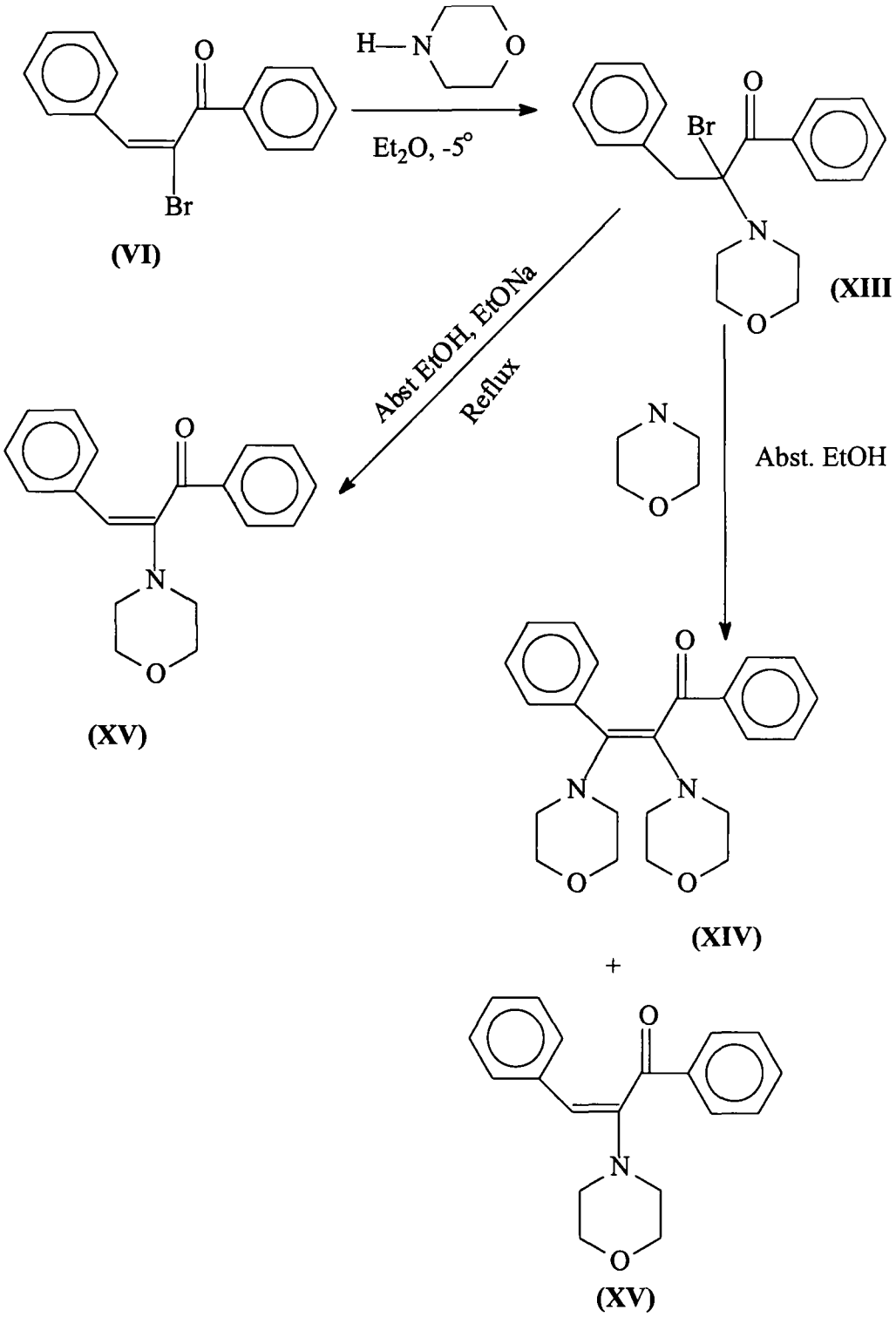
(XI)

+

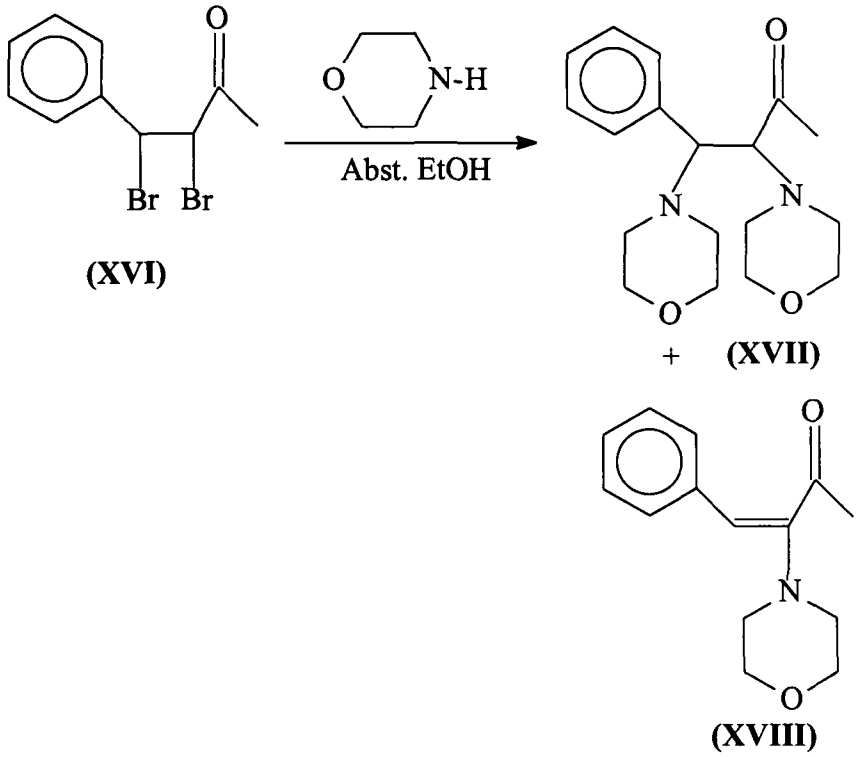
(XII)

When α-bromobenzalacetophenone (VI) was treated with morpholine in the cold, the intermediate, α-bromo-α-morpholinobenzylacetophenone (XIII) was obtained. Compound (XIII) was found to give a slow reaction with morpholine, resulting in the formation of approximately equal amounts of

(XIV) and (XV). However, with sodium ethoxide, 96 % yield of (XV) was obtained[9].

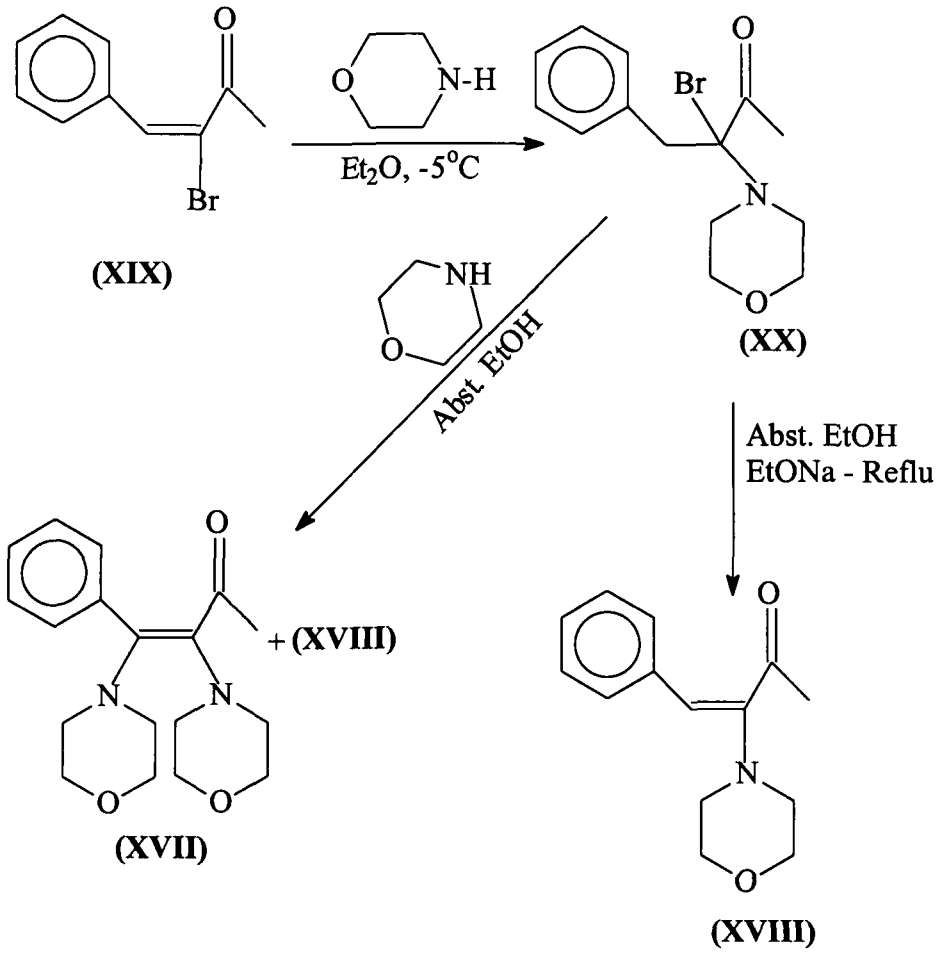


Benzalacetone dibromide (XVI) was found to react rapidly with morpholine to give α,β-dimorpholinobenzalacetone (XVII) with small amounts of α-morpholinobenzalacetone (XVIII) [10].

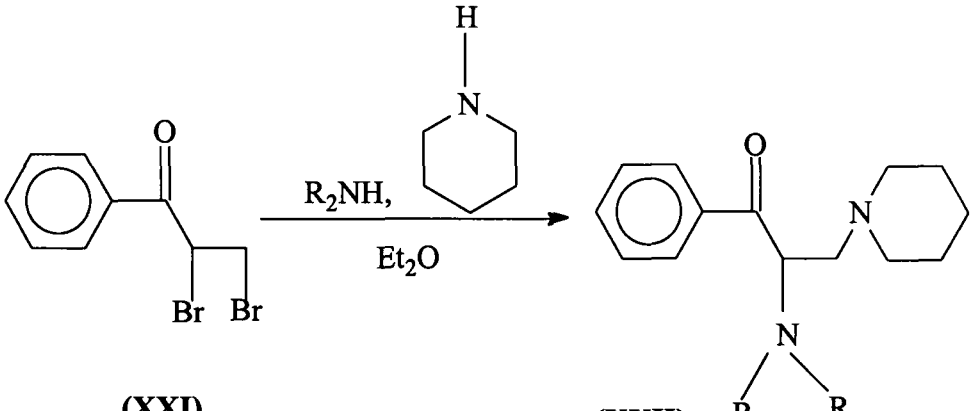


(XVI) **(XVII)** **(XVIII)**

Under special conditions α-bromobenzalacetone (XIX) reacted with morpholine to give α-bromo-α-morpholinobenzylacetone (XX). Compound (XX) reacted slowly with morpholine to give mainly (XVII) and traces of (XVIII). In the usual manner, with sodium ethoxide affords compound (XVIII) in 76% yield[10].



H. L. Davis[11] isolated β-N-piperidino-α-dialkylaminopropiophenones (XXIIa-e) as their dihydrobromides from the reaction of α,β-dibromopropiophenone (XXI) with di-N-alkylamines and with piperidine successively.

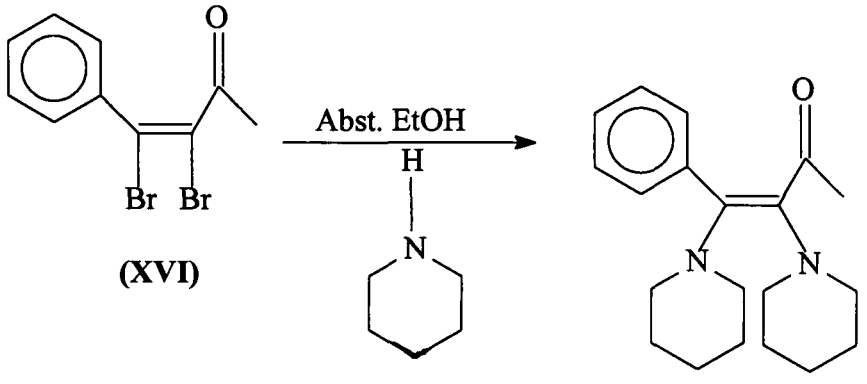


(XXI) **(XXII)**

R

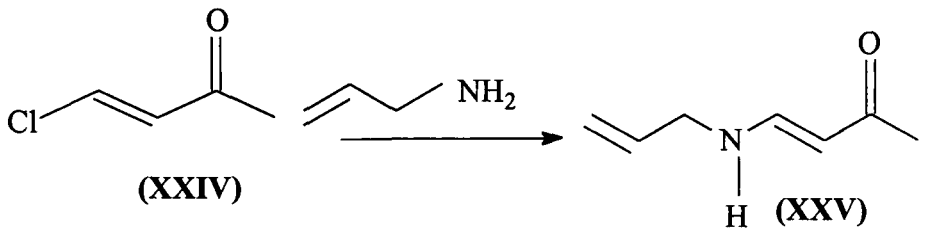
(a) CH_3
(b) C_2H_5
(c) $n\text{-}C_3H_7$
(d) $n\text{-}C_4H_9$
(e) $n\text{-}C_5H_{11}$

Moreu[12] reported the reaction of α,β-dibromobenzylacetone (XVI) with piperidine to give α,β-dipiperidino-benzylacetone (XXIII).



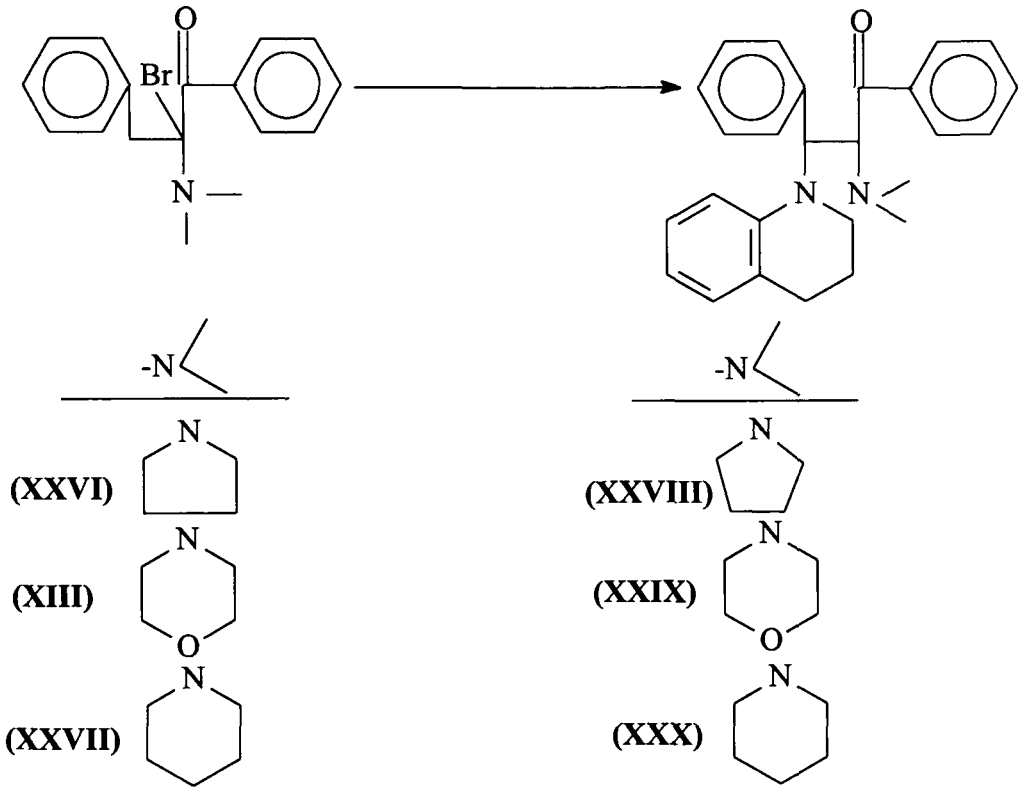
(XVI) **(XXIII)**

Bataafsche[13] prepared aminomethylene ketones by reaction of ammonia or amines with β-halovinyl ketones. 1-Chloro-1-buten-3-one (XXIV) in absolute ether at O°, reacted with allylamine to give 1-allylamino-1-buten-3-one (XXV).

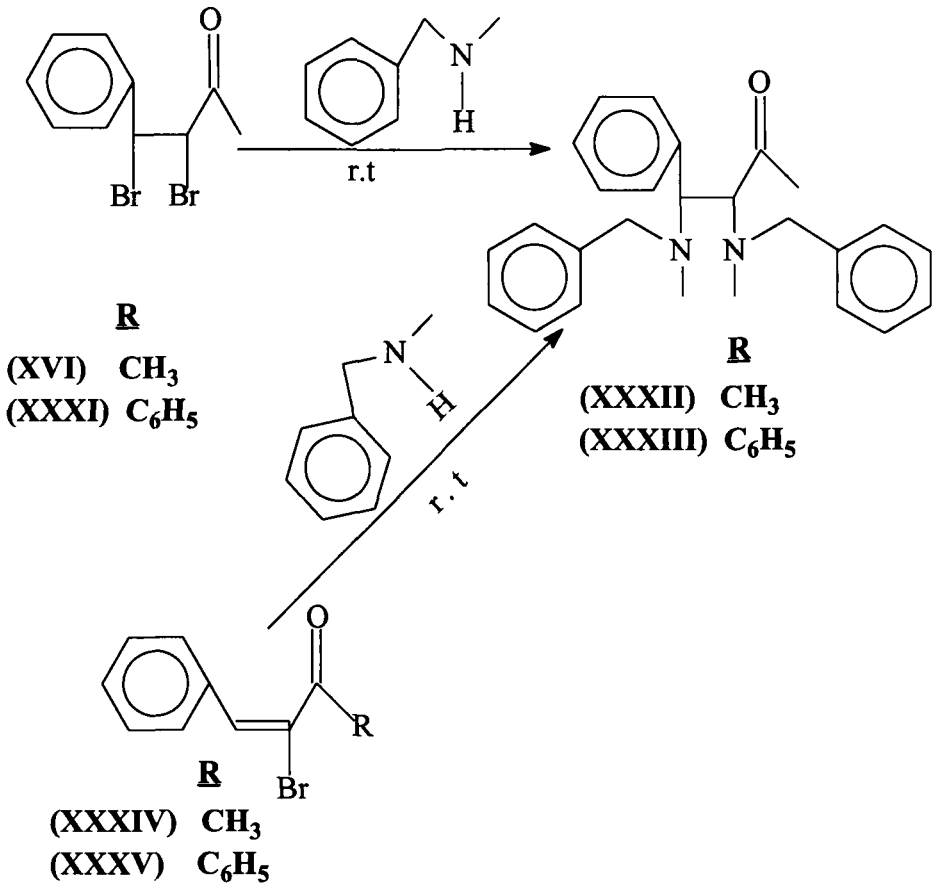


(XXIV) **(XXV)**

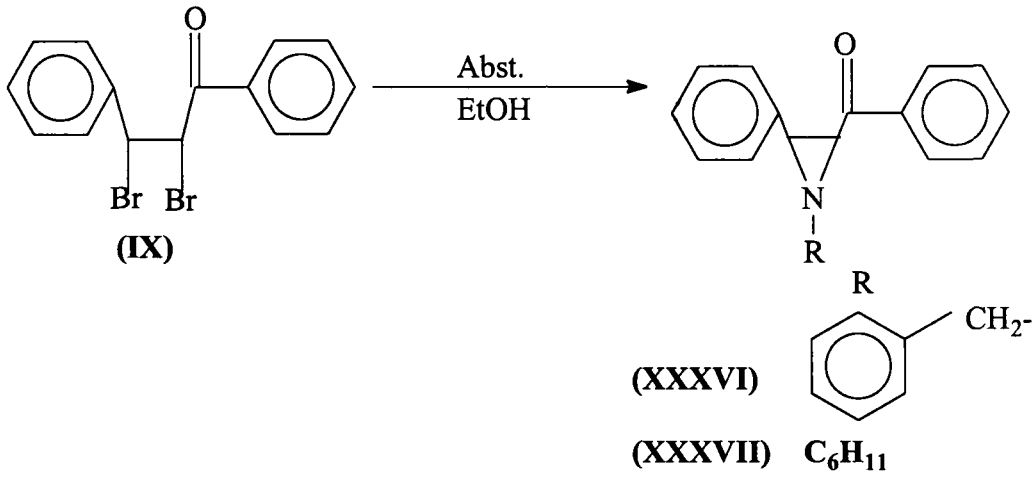
When α-bromo-α-aminoketones (XXVI, XIII, XXVII) were each treated with 2 equivalents of tetrahydroquinoline, the corresponding α,β-diaminoketones (XXVIII – XXX) were obtained in good yields[10].



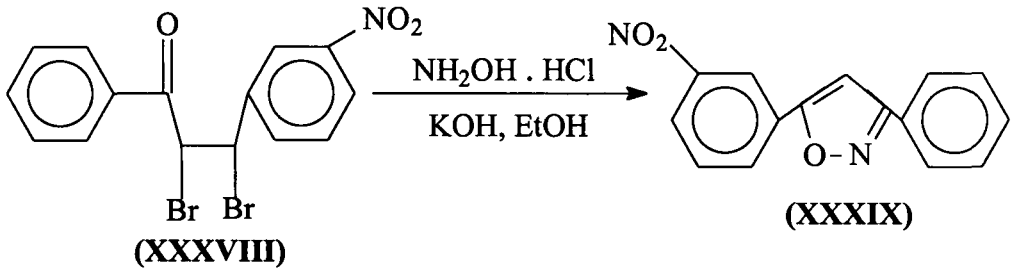
Cromwell and Witt[14] synthesized exclusively α,β-di-N-methylbenzylaminobenzylacetone (XXXII – XXXIII) by treatment of α,β-dibromobenzylacetone (XVI, XXXI) or α-bromobenzalacetone (XXXIV – XXXV) with N-methylbenzylamine at room temperature .



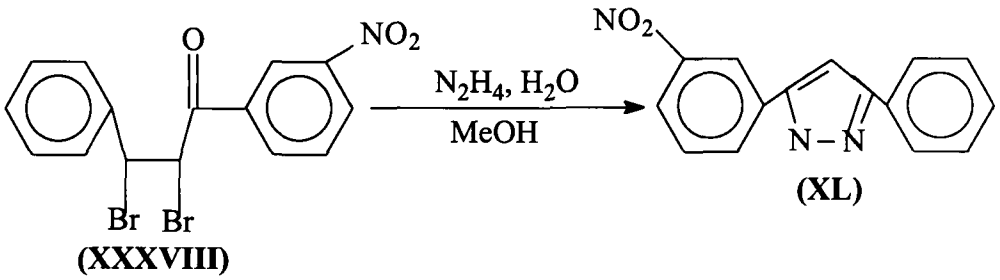
Cromwell et al.[15] suggested that the reaction of benzylamine and cyclohexylamine with α,β-dibromobenzylacetophenone (IX) afforded 1-benzyl-2-phenyl-3- benzoylethylenimine (XXXVI) and 1-cyclohexyl-2-phenyl-3-benzoylethylenimine (XXXVII) respectively.



Barnes and Dodson[16] synthesized 3-m-nitrophenyl-5-phenylisoxazole (XXXIX) by treating m-nitrobenzalacetophenone dibromide (XXXVIII) with hydroxylamine hydrochloride.

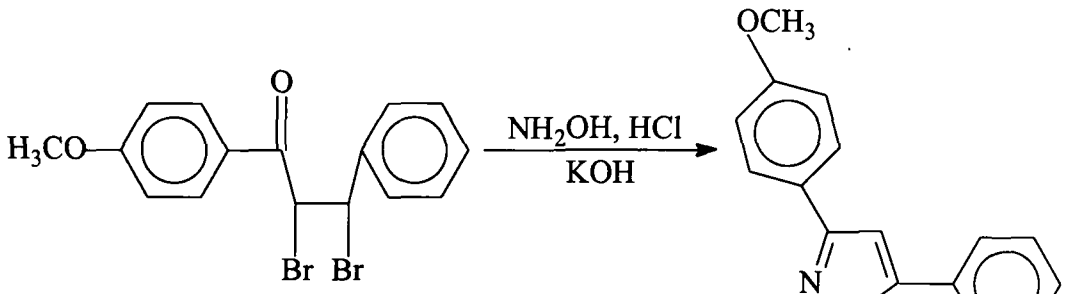


3-m-Nitrophenyl -5-phenylpyrazole (XL) was prepared by the reaction of α,β-dibromobenzal-m-nitroactopheone (XXXVIII) with hydrazine hydrate in methanolic solution[16].



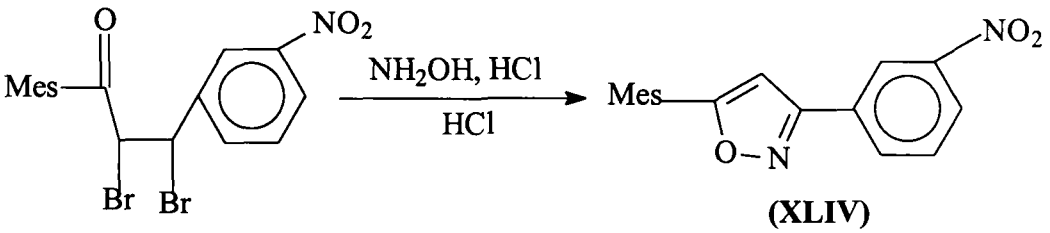
(XXXVIII) **(XL)**

Barnes and Brandon[17] prepared 3-p-methoxyphenyl-5-phenylisoxazole (XLII) by the reaction of benzal –p-methoxyacetophenone dibromide (XLI) with hydroxylamine hydrochloride and potassium hydroxide.



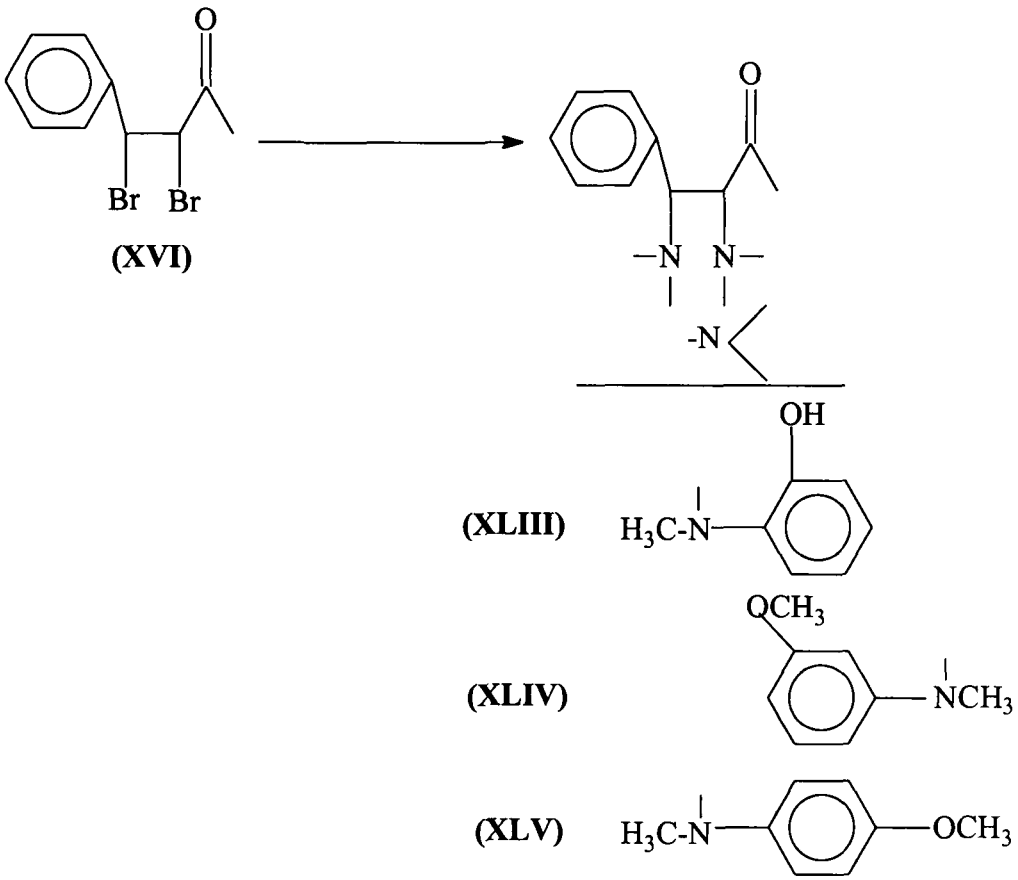
(XLI) **(XLII)**

Barnes and spriggs[18] reported that the reaction of α,β-dibromo-m-nitrobenzalacetomesitylene (XLIII) with hydroxylaminehydrochloride and potassium hydroxide furnished isoxazole (XLIV).



(XLIII) **(XLIV)**

Cromwell and Hoeksema[19] prepared α,β-diaminobenzylacetones (XLIII-XLV) by treating α,β-dibromobenzylacetone (XVI) with o-hydroxy-N-methylbenzylamine, o-methoxy-N-methylbenylamine and p-methoxy-N-methylbenzylamine, respectively.



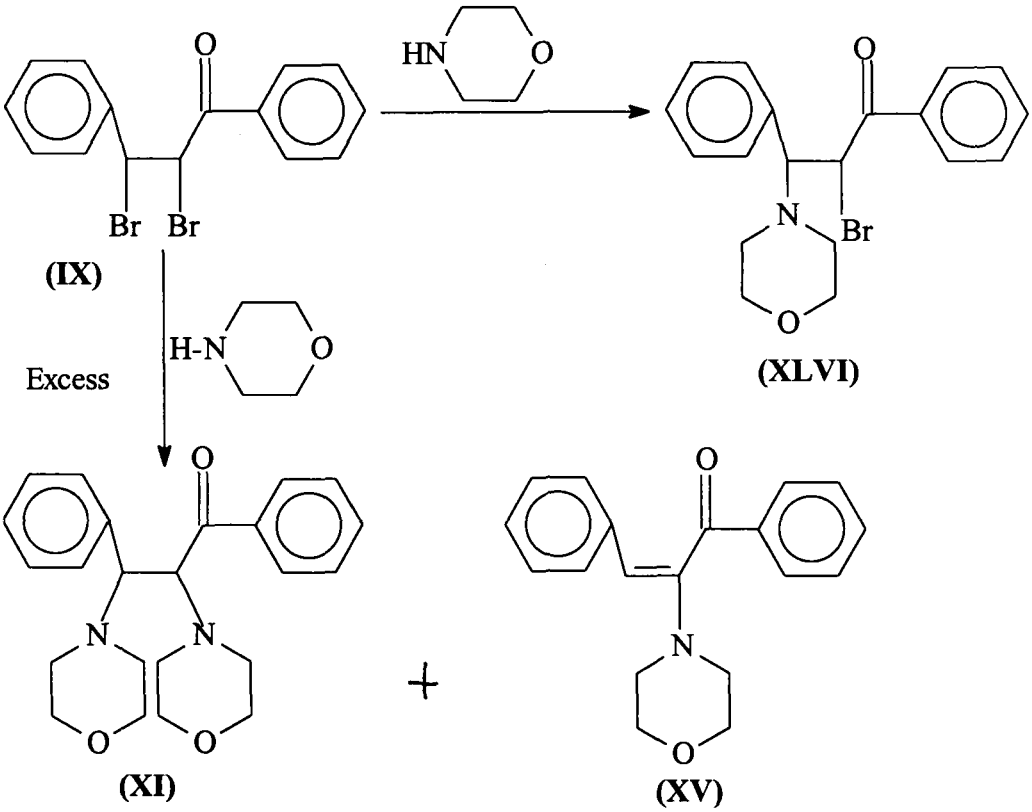
(XVI)

(XLIII)

(XLIV)

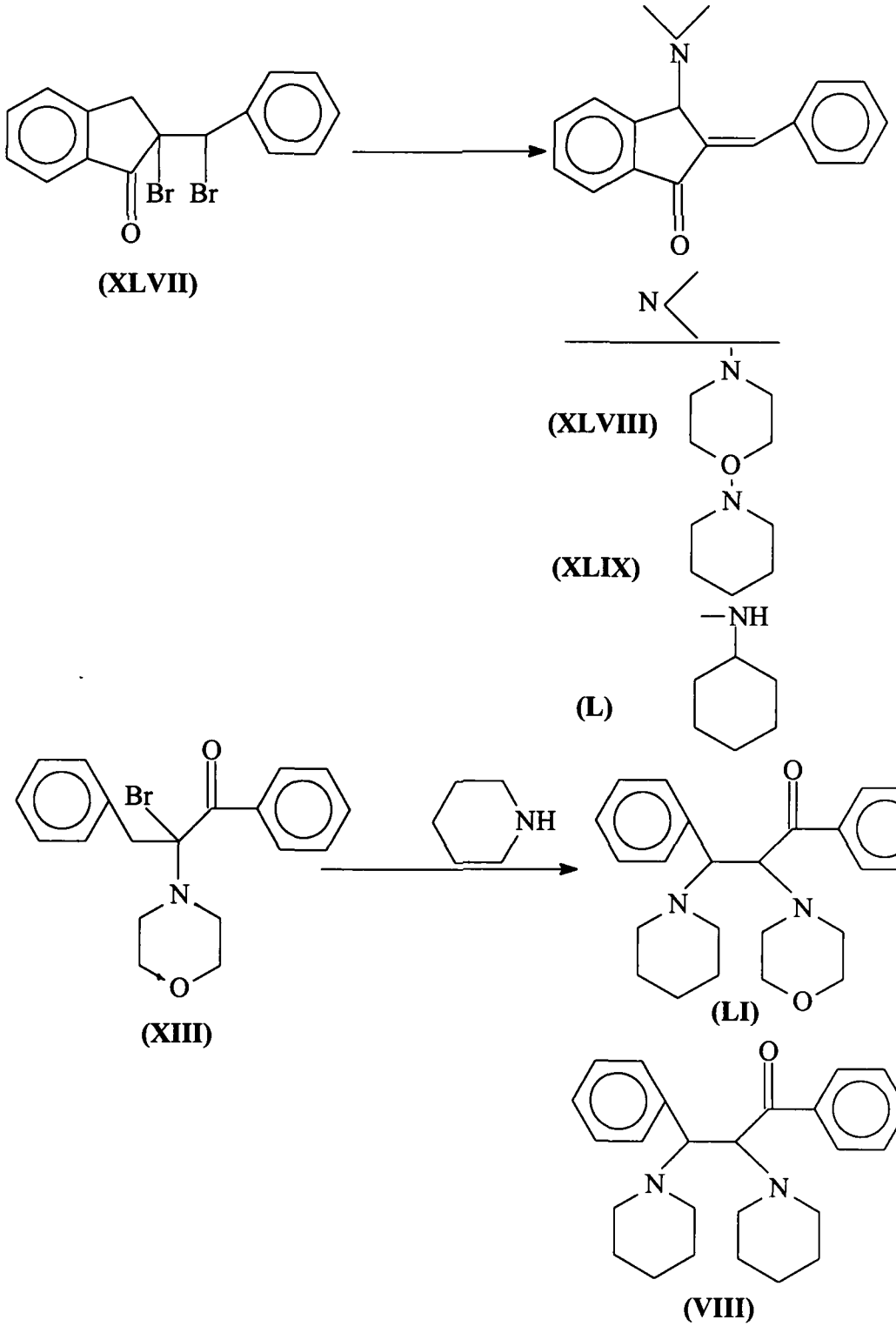
(XLV)

Lutz et al.[20] synthesized α-bromo-β-morpholinobenzylacetophenone (XLVI) and α,β-dimorpholinobenzylacetophenone (XI) and α-morpholinobenzalacetophenone (XV) by the reaction of benzalacetophenonedibromide (IX) with morpholine.

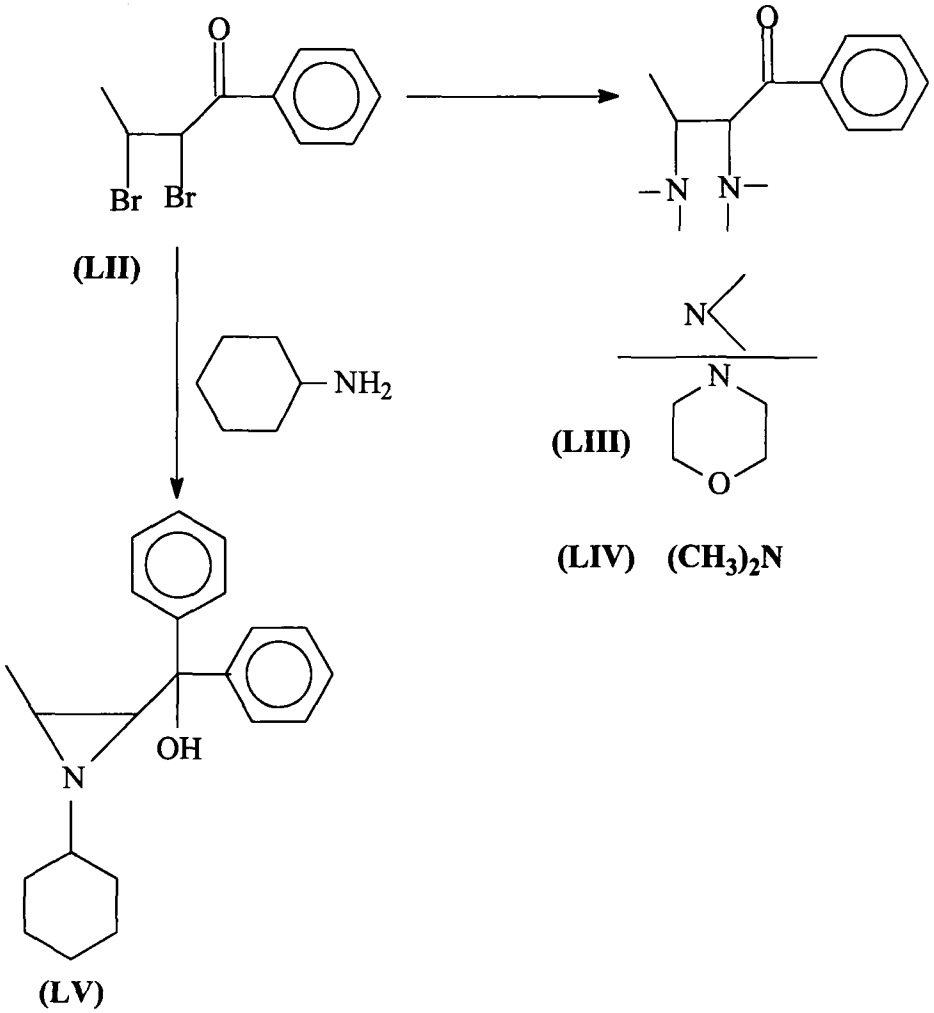


Pearson et al.[21] carried out the reaction of 2-bromo-2-(-α-bromobenzyl)-1-indanone (XLVII) with morpholine, piperidine and cyclohexylamine to give 3-amino-2-benzal-1-indanones (XLVIII-L), whereas 2-bromo-2-(α-bromobenzyl)-3,3-dimethyl-1-indanone (XIII) reacted with

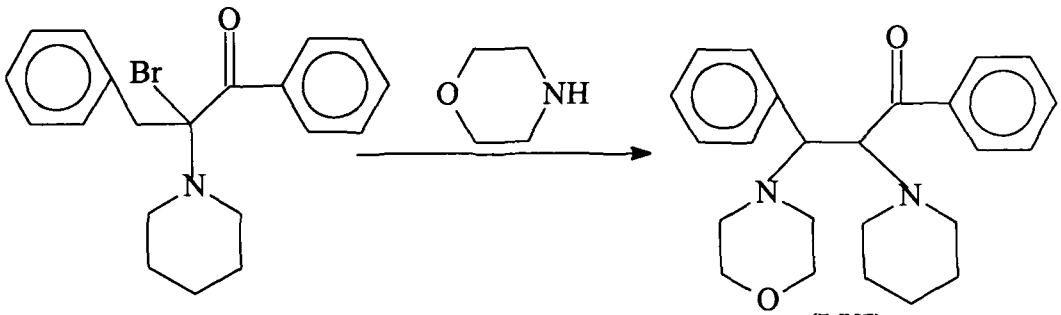
morpholine and piperidine to give β-amino-α,β-unsaturated ketones (LI and VIII).



Sculley and Cromwell[22] investigated the reaction of α,β-dibromobutyrophenone (LII) with morpholine, dimethylamine and cyclohexylamine to furnish α,β-dimorpholinobutyrophenone (LIII), α,β-bis(dimethylamino) butyrophenone (LIV) and 1-cyclohexyl, 2-methyl-4,4-diphenyl-4-hydroxypropyleneimine (LV).

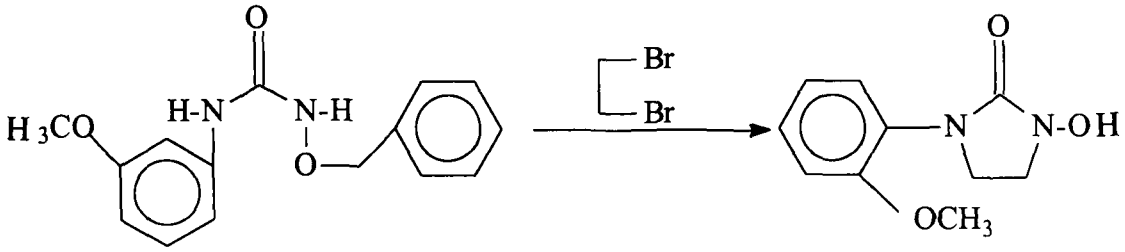


The reaction of α-bromo-α-piperidinobenzylacetophenone (XXVII) with morpholine was reported[23] which was shown to give mainly α-piperidino-β-morpholinobenzylacetophenone (LVI).



(XXVII) **(LVI)**

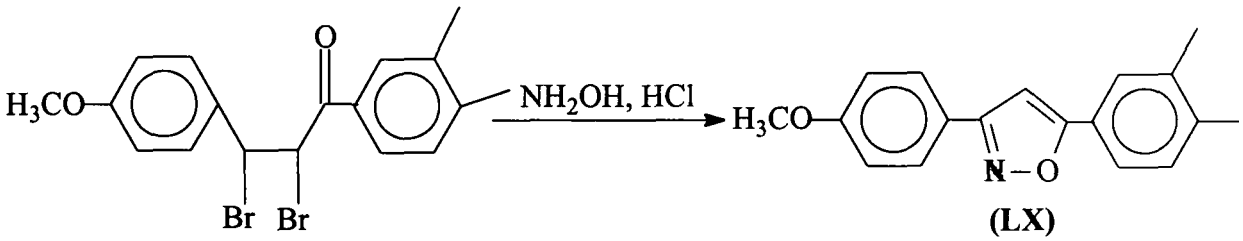
Sulsky and Demers[24] suggested that 2-methoxyphenyl-N-benzyloxyurea (LVII) was alkylated with 1, 2-dibromoethane and subsequently deprotonated to provide 1-hydroxy-3-methoxyphenyl-imidazolidinone (LVIII).



(LVII) **(LVIII)**

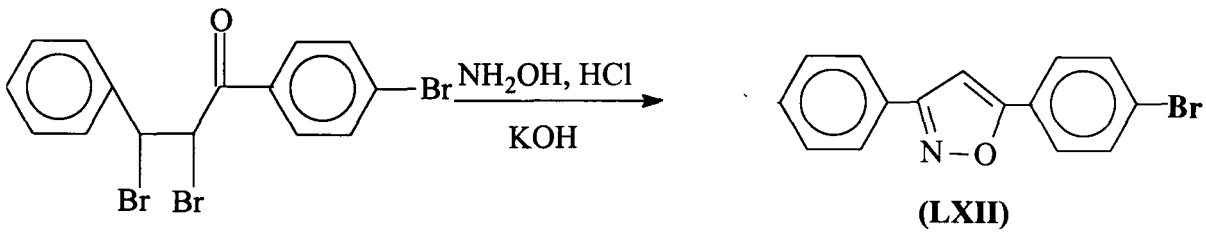
Moussa and Chabaka[25] reported the formation of 3-(m,p-dimethyl) phenyl-5-p-methoxyphenylisoxazole (LX) by the cyclocondensation of α, β-

dibromo-p-methoxybenzal-3, 4-dimethylacetophenone (LIX) with hydroxylamine hydrochloride.

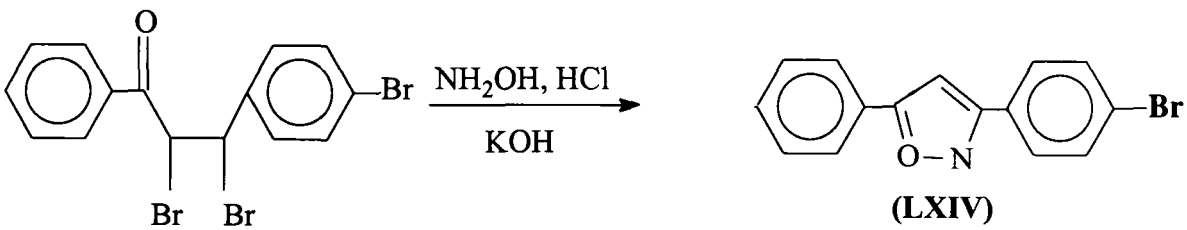


(LIX) **(LX)**

Barnes and Dodson[26] isolated 3-phenyl-5-p-bromophenyl-isoxazole (LXII) from the reaction of α,β-dibromobenzal-p-bromoacetopenone (LXI) with hydroxylamine hydrochloride and potassium hydroxide. The dibromide (LXIII) yielded the isoxazole (LXIV) when reacting with $NH_2OH.HCl$ in alkaline solution.



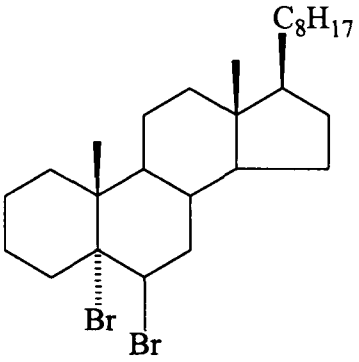
(LXI) **(LXII)**



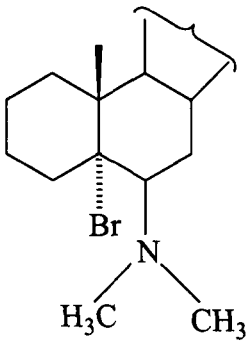
(LXIII) **(LXIV)**

DISCUSSION

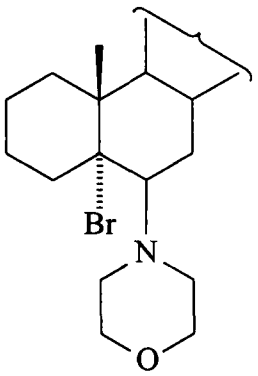
The manifold physiological properties associated with a variety of compounds containing hetero atoms with useful therapeutic values prompted us to carry extensive research in this field. Steroids a class of biologically active compounds were modified to a variety of oxygen and nitrogen containing derivatives, playing a vital role in the era of medicine and drugs and synthetic organic chemistry. These compounds were found to posses dermatological[27], opthalmic[27], antiulcer[28], immunoassay[29] and CNS depressant[30] activities in association with other physiological activities. The present work describes the reaction of 5α, 6β-dibromosteroids (LXV) and 3 keto-5α, 6β-dibromosteroids (LXVI) with dimethylamine, succinimide and morpholine. The structure of these compounds was determined on the basis of analytical and spectral evidences.



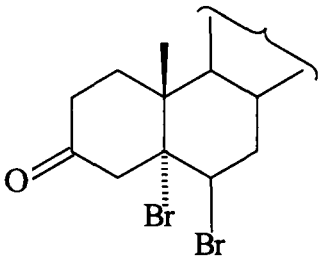
(LXV)



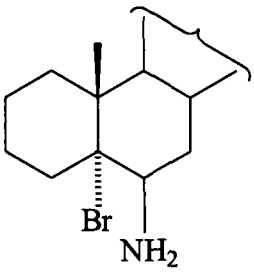
(LXVII)



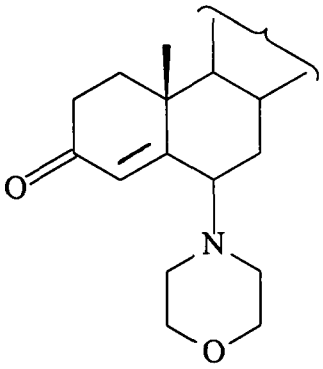
(LXIX)



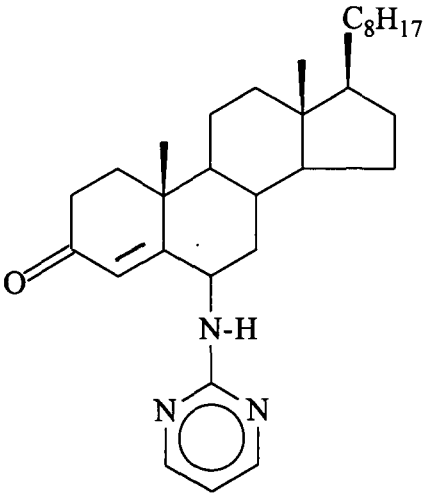
(LXVI)



(LXVIII)



(LXX)



(LXXI)

Reaction of 5, 6β-dibromo-5α-cholestane (LXV) with dimethyl amine :

5, 6β-Dibromo-5α-cholestane[31] (LXV) on reaction with dimethylamine followed by usual workup of the reaction mixture and upon crystallization with chloroform-methanol provided clusters of needles, m.p. 155°C.

Characterization of the compound, m.p. 155°C as 5-bromo-6β-dimethylamino-5α-cholestane (LXVII) :

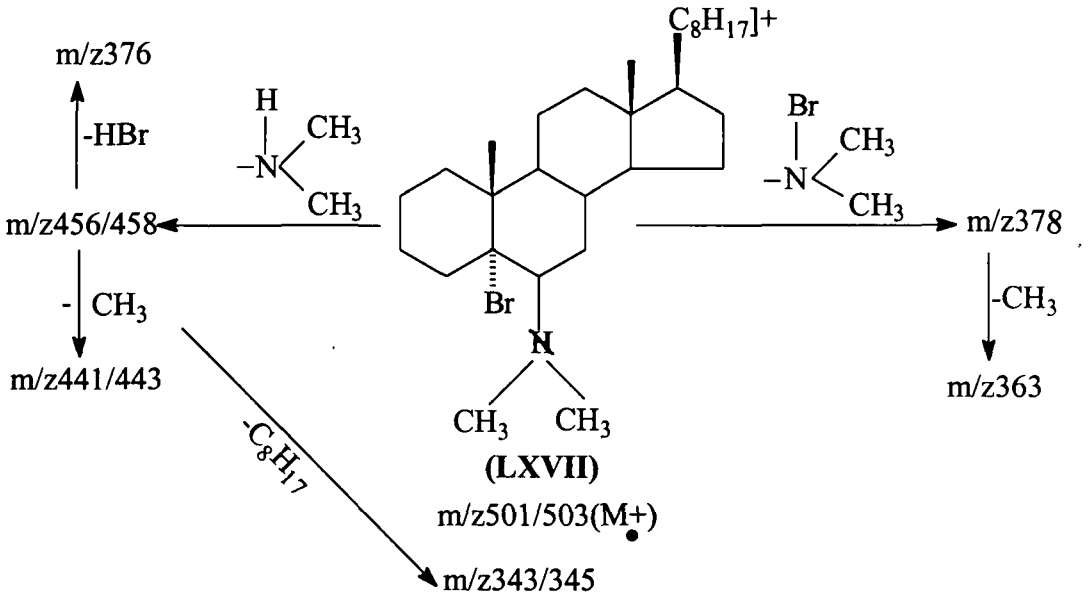
The compound, m.p. 155°C (positive Beilstein's test) was analysed correctly for $C_{29}H_{50}NBr$. The molecular ion peak M^{+} (501/503, 1:1) supports its molecular composition. The I.R spectrum of the compound (LXVII) exhibited absorption band at 1240-1150 for (C-N), 675 cm^{-1} (C-Br)[32]. ^{1}H-NMR spectrum of the compound (LXVII) gave a double doublet at δ 5.0 (1H, Jea = 4.5 Hz and Jee = 2.5 Hz)[33] assigned to H-6α the signal for angular and side chain methyl protons were appeared at δ 1.18 (C10-CH_3). δ 0.68 (C13-CH_3), 0.95, 0.92 and 0.90 other side chain methyl protons. The ^{13}C-NMR spectrum showed signals tabulated in Table – 1

Table – 1

Cabron	δC	Carbon	δC
C 1	36.0931	C 14	55.9930
C 2	24.0215	C 15	25.1258
C 3	27.9943	C 16	39.7955
C 4	41.3478	C 17	54.7526
C 5	86.8666	C 18	11.8962
C 6	56.0240	C 19	19.6236
C 7	36.7267	C 20	35.6908
C 8	31.0322	C 21	18.6137
C 9	45.3663	C 22	24.2136
C 10	49.2543	C 23	23.8033
C 11	21.3535	C 24	39.4618
C 12	28.1507	C 25	28.3168
C 13	42.5792	C 26	22.2330
		C 27	22.5458

The final support for the structure of the compound (LXVII) comes from mass spectral study. The mass spectrum of the compound (LXVII) gave

molecular ion peaks at 501/503 (M^{+}, 1:1) followed by other significant peaks at m/z 456/458, m/z 441/443, m/z 378, m/z 376, m/z 363 and m/z 343/345. Genesis for the formation of these fragment ions is given in scheme – 1.



Scheme - 1

Reaction of 5, 6β-dibromo-5α-cholestane(LXV) with succinimide :

5, 6β-Dibromo-5α-cholestane (LXV) was treated with succinimide in the usual manner. The product on crystallization afforded needles like crystals, m.p. 157°C.

Characterization of the compound, m.p. 157°C, as 5-bromo-6β-amino-5α-cholestane (LXVIII) :

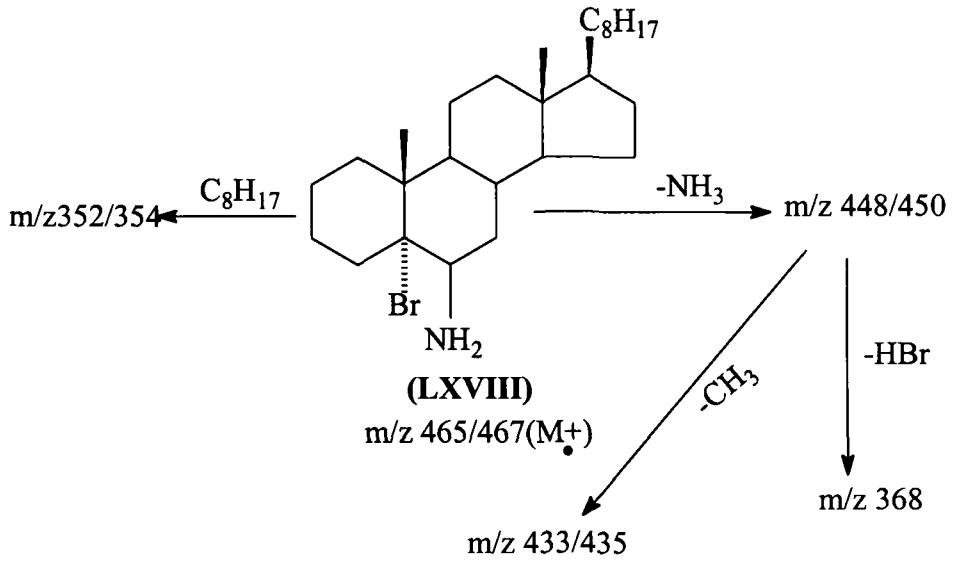
The compound, m.p. 157° was analysed for $C_{27}H_{48}NBr$ (positive Beilstein test). The I.R. spectrum of the compound showed bands at 3450-3430 (–NH), 1220-1120 (C-N) and 675 cm^{-1} (C-Br). In ^{1}H-NMR spectrum of the compound -NH protons appeared as broad singlet at δ 8.2 (exchangeable with deuterium)[32], 4.99 (dd, 1H, Jae = 4.5 Hz and Jee = 2 Hz, H-6α), Angular and side chain methyl protons appeared at δ 1.2 (C10-CH_3), 0.65 (C13-CH_3), 0.90 and 0.85 (side chain methyl protons). ^{13}C-NMR spectrum of the compound (LXVIII) gave δ_C values for various carbons as follows given in table – 2.

Table – 2

Carbon	δC	Carbon	δC
C 1	36.7125	C 15	24.0098
C 2	31.0195	C 16	39.7829
C 3	27.9801	C 17	56.5356
C 4	42.5666	C 18	11.8836
C 5	86.8304	C 19	21.3392
C 6	56.0097	C 20	35.6765

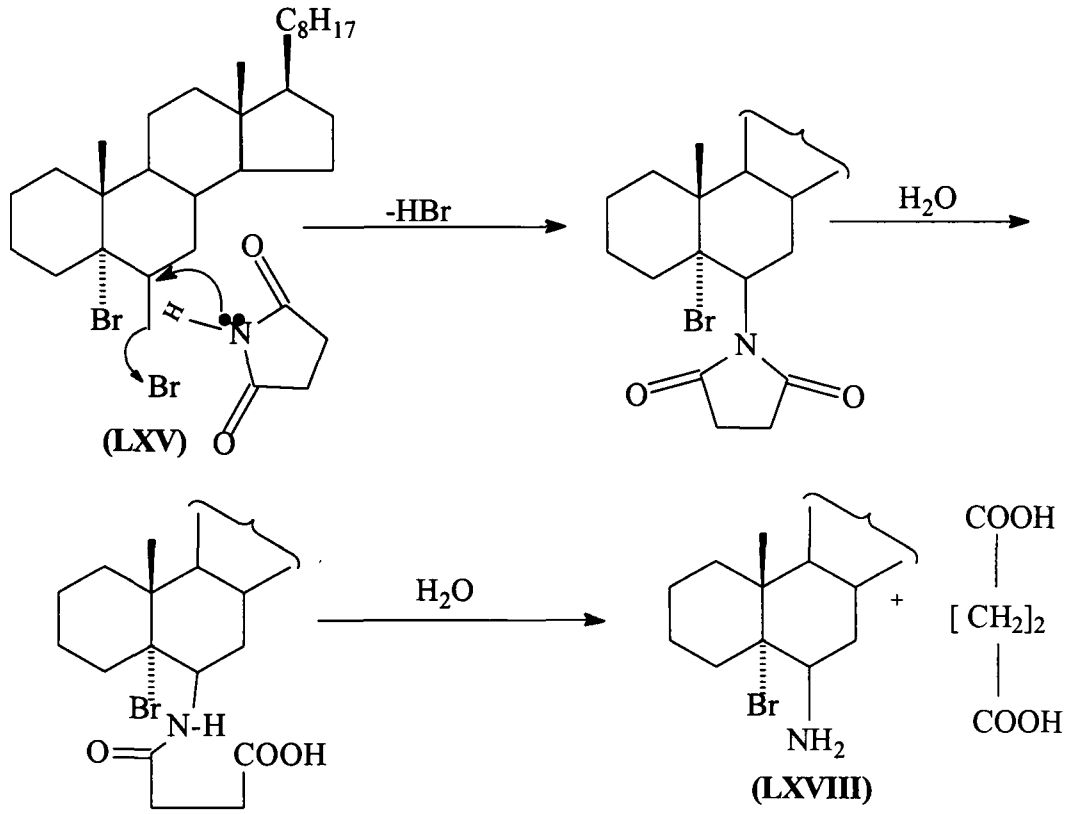
C 7	39.4555	C 21	18.6059
C 8	31.0195	C 22	36.0853
C 9	45.3520	C 23	23.7922
C 10	41.3352	C 24	39.0853
C 11	21.3392	C 25	28.1413
C 12	28.1413	C 26	22.5213
C 13	42.0845	C 27	22.7921
C 14	55.9840		

The final support of the structure of the compound (LXVIII) comes from its mass spectral study. The mass spectrum of the compound (LXVIII) gave molecular ion peaks at m/z 465/467 ($M^{+\bullet}$, 1:1) followed by some significant fragment ion mass peaks such as m/z 448/450, m/z 433/435, m/z 368, m/z 352/354. The genesis of few of them is given below in scheme - 2



Scheme - 2

A tentative mechanism for formation of 5-bromo-6β-amino-5α-cholestane may be given as :



Reaction of 5, 6β-dibromo-5α-cholestane(LXV) with morpholine :

5, 6β-Dibromo-5α-cholestane (LXV) reacted with morpholine with stirring for 1 hr. After keeping the reaction mixture at room temperature for 3 days, it was worked up in the usual manner and chromotographed over silica gel to provide a solid crystalline compound, m.p. 160°C.

Characterization of compound, m.p. 160°C as 5-bromo-6β-morpholino-5α-cholestane (LXIX) :

The compound, m.p. 160°C (positive Beilstein's test) was analysed correctly for $C_{31}H_{53}NOBr$. The I.R. spectrum of the compound showed bands at 1185-1150 (C-N), 1075(C-O) and 670 cm^{-1} (C-Br). ^{1}H-NMR spectrum of the compound (LXIX) gave a double doublet at δ 5.1 (dd, 1H, Jae = 4.4 Hz and Jee = 2.5 Hz) for one proton was attributed due to H-6α, a multiplet at δ2.4 to 2.1 integrating for four protons assigned to two methylene group adjacent to nitrogen atom of morpholine group, another multiplet centered at δ3.7 for four protons was assigned to two methylene group adjacent to oxygen atom of morpholino group. Angular and side chain methyl protons appeared at δ 1.18 (C10-CH_3), 0.65 (C13-CH_3), 0.93 and 0.85 (side chain methyl protons).

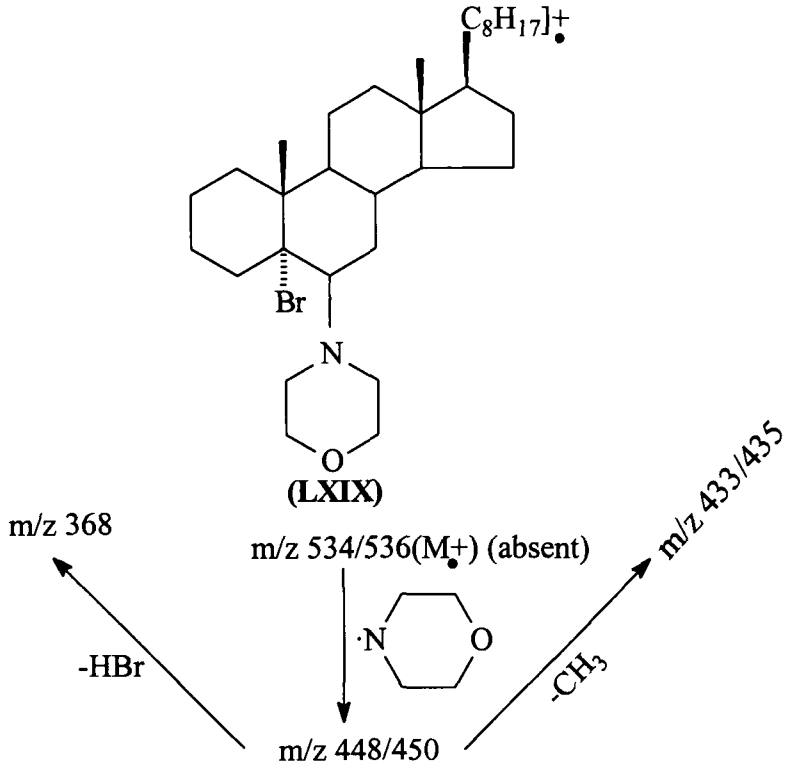
^{13}C-NMR spectrum of the compound (LXIX) gave δ_C values of various carbon atoms tabulated in table – 3

Table – 3

Carbon	δC	Carbon	δC
C 1	36.0912	C 16	39.4598
C 2	24.0132	C 17	56.5825
C 3	27.9860	C 18	11.8895
C 4	41.3411	C 19	19.6135
C 5	86.8436	C 20	35.6624
C 6	55.9863	C 21	18.6118
C 7	36.7200	C 22	36.5126
C 8	31.0254	C 23	23.7981
C 9	45.3579	C 24	39.8126
C 10	42.0904	C 25	28.1456
C 11	21.3451	C 26	22.5455
C 12	28.1456	C 27	22.7964

C 13	42.5725
C 14	57.1285
C 15	23.7981

The structure of the compound (LXIX) was finally supported by its mass spectral studies. In the mass spectrum, the molecular ion peaks at m/z 534/536 is absent but other significant fragment ion peaks at m/z 448/450, m/z 433/435 and m/z 368 with their genesisaregiven in scheme – 3.



Scheme - 3

Reaction of 5, 6β-dibromo-5α-cholestan-3-one (LXVI) with morpholine :

5, 6β-Dibromo-5α-cholestan-3-one[31] (LXVI) was reacted with morpholine with stirring for 1 hr. After keeping the reaction mixture at room temperature for 3 days, it was worked up in the usual manner and chromatography over silica gel to provide a solid crystalline compound, m.p. 125°.

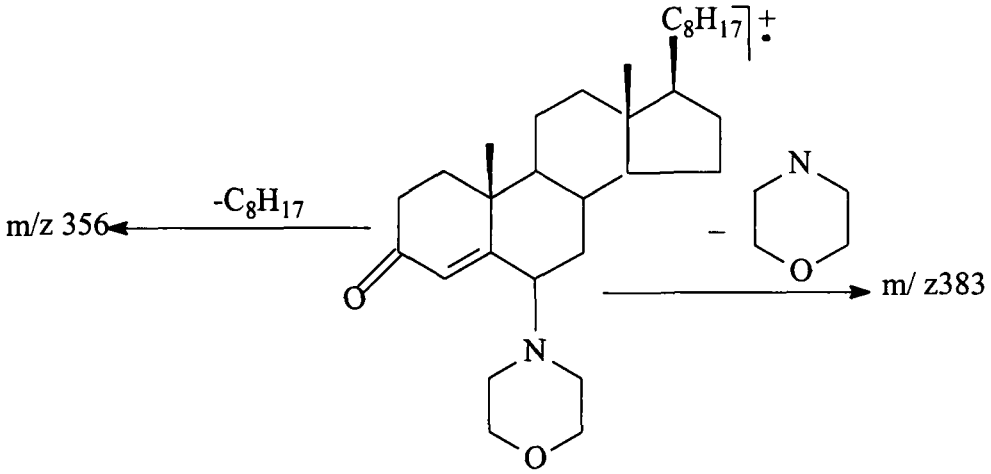
Characterization of the compound, m.p. 125° as 6β-morphilono-cholest-4-en-3-one (LXX) :

The compound (LXX), m.p. 125°C (negative Beilstein test) was analysed correctly for $C_{31}H_{50}NO_2$. The I. R. spectrum of the compound (LXX) gave bands at 1690 (C=C-C=O), 1605 (C=C), 1185, 1120 cm^{-1} (C-N). ^{1}H-NMR spectrum gave peaks at δ 6.16 (s, H-4, olefinic proton), 5.2 (dd, 1H, Jae = 4.5 Hz, Jee = 2.5 Hz, H-6α), angular and side chain methyl protons appeared at δ 1.15 (C10-CH_3), 0.70 (C13-CH_3), 0.95 and 0.90 (side chain methyl protons). δ_C value of various carbons are tabulated in table – 4.

Table - 4

Carbon	δ C	Carbon	δ C
C 1	36.0962	C 14	57.2135
C 2	23.9285	C 15	23.7623
C 3	199.4194	C 16	39.4223
C 4	125.3974	C 17	56.1285
C 5	161.0208	C 18	11.8585
C 6	55.9276	C 19	17.4749
C 7	36.0310	C 20	35.6352
C 8	33.9346	C 21	18.6135
C 9	45.7685	C 22	36.5215
C 10	42.4992	C 23	23.9285
C 11	20.8466	C 24	39.1045
C 12	27.9664	C 25	28.3126
C 13	39.7725	C 26	22.7720
		C 27	22.5179

The structure of the compound (LXX) was further supported by its mass spectral study. The mass spectrum of the compound (LXX) gave molecular ion peak at m/z 469 ($M^{+\bullet}$) followed some significant fragment ion peaks at m/z 454, m/z 383, m/z 356 and lower mass peaks. The genesis of few of them is given in scheme – 4.



(LXX)

m/z 469($M^{+\bullet}$)

$-CH_3$

m/z 454

Scheme - 4

Reaction of 5, 6β-dibromo-5α-cholestan-3-one (LXVI) with 2-aminopyrimidine :

5,6β-Dibromo-5α-cholestan-3-one (LXVI) with 2-aminopyrimidine followed by usual work up of the reaction mixture and upon crystallization with chloroform-methanol provided crystallaline compound m.p. 180-81°C.

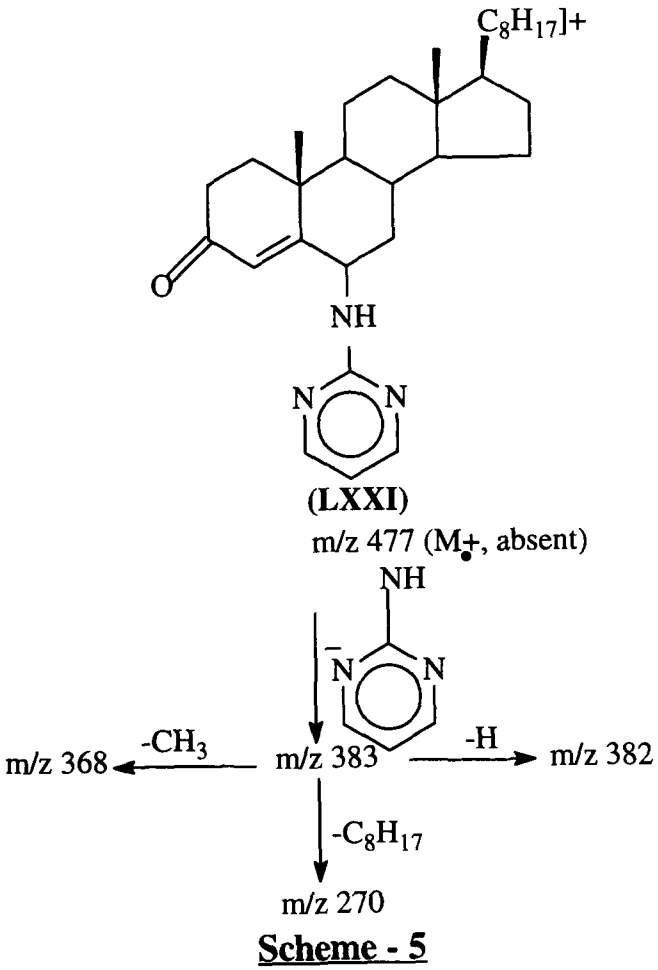
Characterization of the compound, m.p. 180-81°C as 6β-aminopyrimidinocholest-4-en-3-one (LXXI) :

The compound, m.p.180-81° (negative beilestein's test) was analysed correctly as $C_{31}H_{47}N_3O$. The I.R. spectrum of compound (LXXI) gave bands at 3500 - 3450 (-NH), 1690 (>C=O) 1605 (C=C), 1140, 1080 cm^{-1} (C-N). The ^{1}H-NMR spectrum of the compound gave peak at δ 8.35 (brs, 1H, -NH, exchangeable with deuterium), 7.3 (brs, 3H, aromatic protons). 5.4 (slightly resolved s, 1H, H-4 olefinic) 3.76 (mc, 1H, w½ = 7Hz, H-6α, equatorial) 1.1 (C10-CH_3), 0.70 (C13-CH_3), 0.90, 0.82 (side chain methyl protons). ^{13}C-NMR gave δ_C values of various carbons tabulated in the table-5.

Table-5

Carbon	δC	Carbon	δC
C 1	35.7909	C 14	56.1539
C 2	23.8350	C 15	24.279
C 3	145.8456	C 16	39.5275
C 4	42.6136	C 17	56.5238
C 5	141.5633	C 18	11.8595
C 6	56.7170	C 19	18.7269
C 7	36.1949	C 20	36.1436
C 8	33.3997	C 21	19.2644
C 9	50.0908	C 22	35.8526
C 10	43.4189	C 23	24.2136
C 11	20.9747	C 24	39.1269
C 12	28.0277	C 25	28.2183
C 13	39.7725	C 26	22.5677
		C 27	22.8235

The structure of the compound (LXXI) as 6-aminopyrimidinocholest-4-en-3-one (LXXI) was further established by its mass spectral study. The molecular ion at m/z 477 ($M^{+\bullet}$) was absent in the mass spectrum of the compound (LXXI) but other significant fragment ion peaks m/z 383, m/z 382, m/z 368, m/z 270 and lower mass peaks were obtained, the genesis of which was given as below in scheme – 5.



Scheme - 5

EXPERIMENTAL

5, 6-Dibromo-5α-cholestane (LXV) :

To a solution of cholest-5-ene (LXXII) (3.5 g) in dry ether (25 ml) was added gradually bromine solution (2.4 g) in glacial acetic acid (25 ml) containing anhydrous sodium acetate (250 mg) with stirring. The solution turned yellow and promptly set to a paste of the dibromide. The mixture was cooled to 20°C and stirred with a glass rod for 5 minutes to ensure complete crystallization. The product was then collected by filteration under suction and washed with cold ether-acetic acid (3:7) mixture until the filterate was completely colourless.

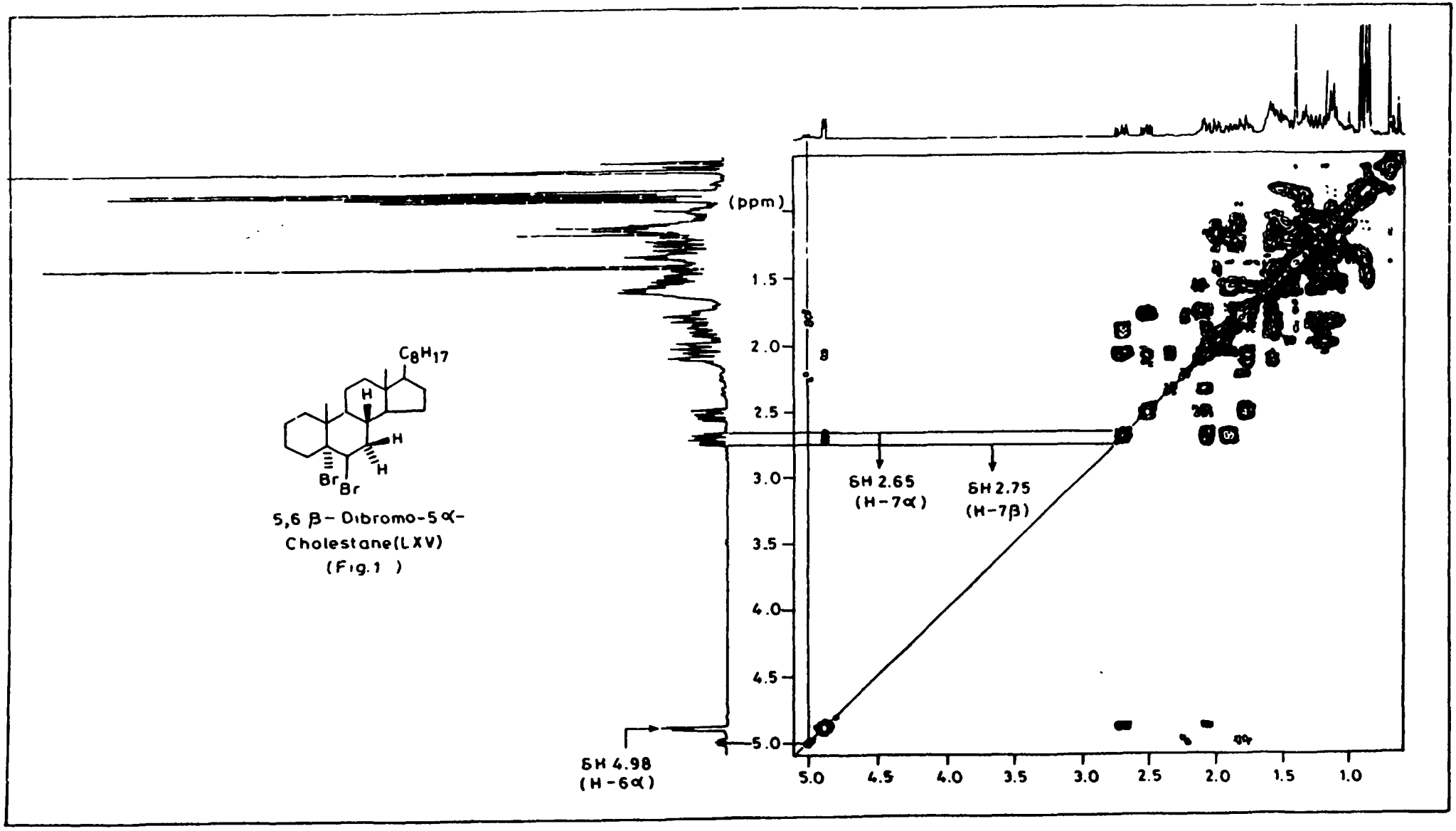
5,6β-Dibromo-5α-cholestane (LXV) :

Solvent of crystallization : acetone, Yield : (3.4 g), m.p. 111° (reported[31] m.p. 110-111°) (positive Beilstein test.)

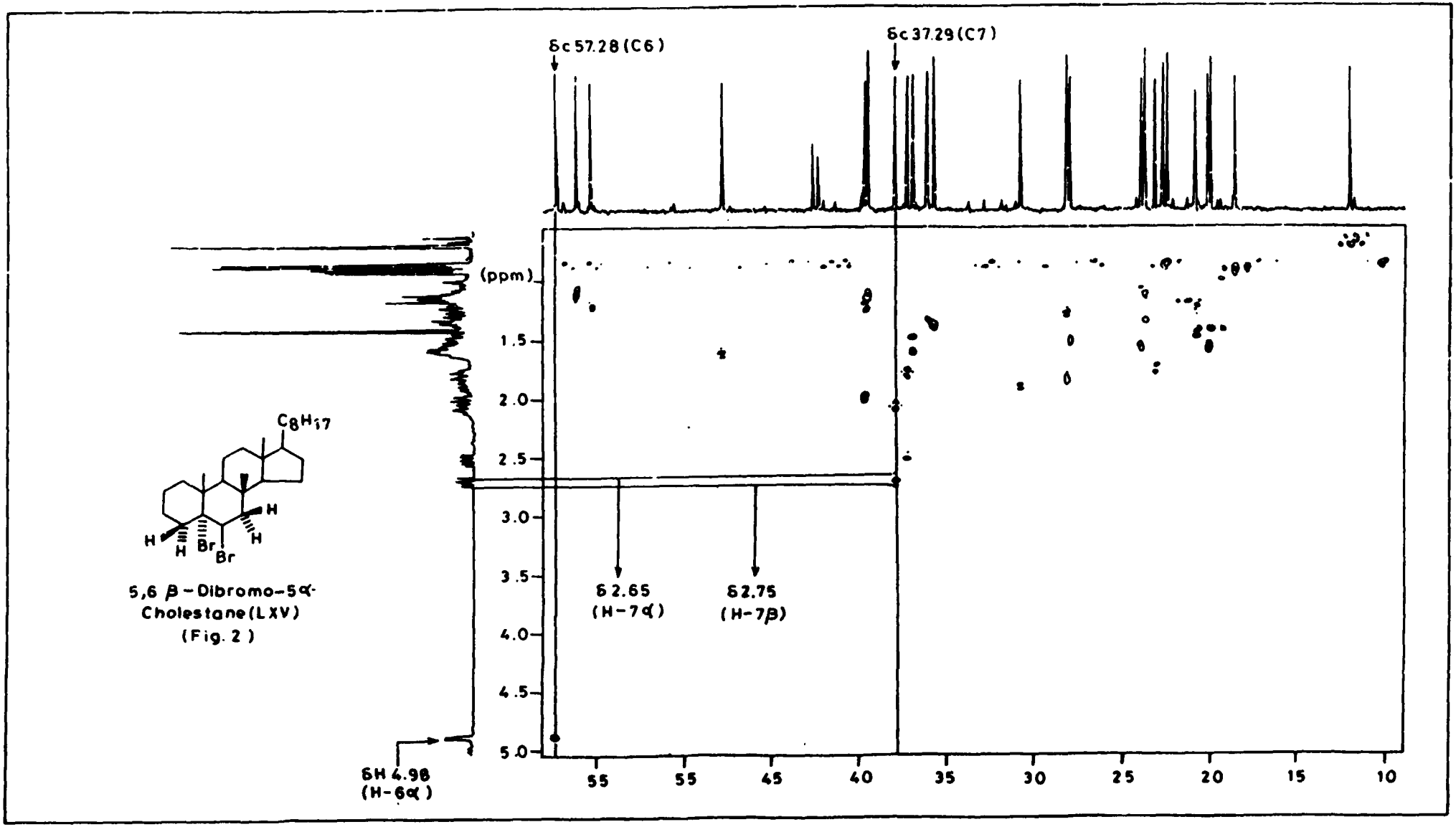
Analysis found : C, 61.14; H, 8.67

$C_{27}H_{46}Br_2$ requires : C, 61.13; H, 8.68%

I.R : ν max 1475 ($-CH_2-$ bending), 670 cm-1 (C-Br).



5,6 β– Dibromo-5α-
Cholestane(LXV)
(Fig.1)


δc 57.28 (C6)
δc 37.29 (C7)
(ppm)
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
δ 2.65
(H-7α)
δ 2.75
(H-7β)
δH 4.98
(H-6α)
55
55
45
40
35
30
25
20
15
10
C8H17
H
H
Br
Br
H
5,6 β-Dibromo-5α-
Cholestane (LXV)
(Fig. 2)

^{1}H-NMR ($CDCl_3$) : δ 4.98 (dd, 1H, J = 4.5 Hz and 2.0 Hz, H-6α), 1.4 (C10-CH_3), 0.70 (C13-CH_3), 0.91, 0.87 and 0.85 (side chain methyl group).

^{13}C-NMR : δ_c 39.075 (C-1), 23.24 (C-2), 20.94 (C-3), 36.98 (C-4), 93.25 (C-5), 57.28 (C-6), 37.29 (C-7), 30.82 (C-8), 47.81 (C-9), 42.42 (C-10), 20.20 (C-11), 28.17 (C-12), 42.68 (C-13), 55.31 (C-14), 24.02 (C-15), 39.68 (C-16), 56.12 (C-17), 12.20 (C-18), 20.07 (C-19), 35.76 (C-20), 18.66 (C-21), 36.15 (C-22), 23.81 (C-23), 39.51 (C-24), 28.00 (C-25), 22.55 (C-26), 22.81 (C-27).

^{1}H-^{1}H-NMR homonuclear and ^{1}H-^{13}C-NMR heteronuclear cosy spectrum were also studied. H-6α proton appeared as double doublet was coupled with H-7α at δ 2.65 (Jea = 4 Hz) and H-7β at δ 2.75 (Jee = 2.0 Hz), δ 4.98 (H-6α) is correlated with δ_C 57.28 (C6 – one bond correlation) and δ 2.65 (H-7α) and δ (H-7β) to δ_C 37.29 (C7) Fig. 1 and 2.

Mass m/z 528/530/532 (1:2:1; M+).

3β-Hydroxy-5, 6β-dibromo-5α-cholestane (LXXII) :

To a solution of cholesterol (14 g) in ether (100 ml) was added gradually the bromine solution (9.6 g in 100 ml of glacial acetic acid containing 1 g of anhydrous sodium acetate). The solid thus obtained was filtered under suction and washed with cold ether-acetic acid mixture (3:7). The dried dibromide (16 g) showed m.p. 113-114° (reported[31], m.p. 114°).

5, 6β-Dibromo-5α-cholestan-3-one (LXVI) :

3β-Hydroxy-5, 6β-dibromo-5α-cholestane (LXVI) (10 g) was suspended in acetone (300 ml). The suspension was cooled to 0.5°. It was stirred for 5 min. and to this mixture Jone's reagent was added drop wise over a period of 20 min. at the maintained temperature of 0-5°. Water (200 ml) was added and dibromoketone was filtered under suction, washed with water, methanol and air dried (9 g), m.p.73-75° (reported[31], m.p. 73-75°).

Reaction of 5, 6β-dibromo-5α-cholestane (LXV) with dimethyl amine : 5-Bromo-6β-dimethylamino-5α-cholestane (LXVII) :

To a solution of 5, 6β-dibromo-5α-cholestane (LXV) (1.5 g) in benzene extrapure crystallizable (20 ml), dimethylamine (40%) solution (2.98 ml) was added gradually over stirring for half an hour at room temperature. It was allowed to stand for 2 days at room temperature. Solvent was evaporated in vacuum and the residue was redissolved in ether. Ethereal layer was washed successively with water several times, very dilute hydrochloric acid solution water, sodium bicarbonate (5%) and water and dried over anhydrous sodium sulphate.

5-Bromo-6β-dimethylamino-5α-cholestane (LXVII) :

Solvent of crystallization : chloroform-methanol, Yield : (1.29 g), m.p. 155°C (positive Beilstein's test).

Analysis found : C, 70.86; H, 10.17

$C_{29}H_{50}NBr$ requires : C, 70.87; H, 10.18%

IR : ν max 1240 – 1150 (C-N), 675 cm^{-1} (C-Br).

^{1}H-NMR ($CDCl_3$) : δ 5.0 (dd, 1H, Jea = 4.5 Hz, Jee = 2.5 Hz, H-6α),

1.18 (C10-CH_3), 0.68 (C13-CH_3), 0.95, 0.92 and 0.90 (side chain methyl protons).

^{13}C-NMR δ_C 36.0931 (C-1), 24.0215 (C-2), 27.9943 (C-3), 41.3478 (C-4), 86.8666 (C-5), 56.0240 (C-6), 36.7267 (C-7), 31.0322 (C-8), 45.3663 (C-9), 49.2543 (C-10), 21.3535 (C-11), 28.1507 (C-12), 42.5792 (C-13), 55.9930 (C-14), 25.1258 (C-15), 39.7955 (C-16), 54.7526 (C-17), 11.8962 (C-18), 19.6236 (C-19), 35.6908 (C-20), 18.6137 (C-21), 24.2136 (C-22), 23.8033 (C-23), 39.4618 (C-24), 28.3168 (C-25), 22.2330 (C-26), 22.5458 (C-27).

Mass : m/z 501/503 ($M^{+\cdot}$; 1:1), m/z 456/458, m/z 441/443, m/z 378, m/z 376 m/z 363, m/z 343/345.

Reaction of 5,6-dibromo-5α-cholestane (LXV) with succinimide : 5-Bromo-6-amino-5α-cholestane (LXVIII) :

Reaction of 5,6-dibromo-5α-cholestane (LXV)(1.5 g) in benzene (20 ml) with succinimide (0.47 g) was carried out in a manner described earlier. The ethereal layer was washed several times with water, dried over anhydrous sodium acetate. The solvent was evaporated.

5-Bromo-6-amino-5α-cholestane (LXVIII) :

Solvent of crystallization : acetone, Yield : (1.35 g), m.p. 157°, (positive Beilstein test).

Analysis found : C, 70.27; H, 10.40

$C_{27}H_{48}NBr$ requires : C, 70.28; H, 10.41%

IR : ν max 3450 – 3430 (-NH), 1220 – 1120 (C-N), 675 cm^{-1} (C-Br)

^{1}H-NMR ($CDCl_3$) : δ 8.2 (brs, 1H, exchangeable with deuterium, -NH), 4.99 (dd, 1H, Jae = 4.5 Hz, Jee = 2 Hz, H-6α), 1.2 (C10-CH_3), 0.65 (C13-CH_3), 0.90 and 0.85 (side chain methyl protons).

^{13}C-NMR δ_C 36.7125 (C-1), 31.0195 (C-2), 27.9801 (C-3), 42.5666 (C-4), 86.8304 (C-5), 56.0097 (C-6), 39.4555 (C-7), 31.0195 (C-8), 45.3520 (C-9), 41.3352 (C-10), 21.3392 (C-11), 28.1413 (C-12), 42.0845 (C-13), 55.9840 (C-14), 24.0098 (C-15), 39.7829 (C-16), 56.5356 (C-17), 11.8836 (C-18), 21.3392 (C-19), 35.6765 (C-20), 18.6059 (C-21), 36.0853 (C-22), 23.7922 (C-23), 39.0853 (C-24), 28.1413 (C-25), 22.5130 (C-26), 22.7921 (C-27).

Mass : m/z 465/467 ($M^{+\bullet}$, 1:1), m/z 448/450, m/z 433/435, m/z 368, m/z

352/354.

Reaction of 5, 6β-dibromo-5α-cholestane(LXV) with morpholine :

5-Bromo-6β-morpholino-5α-cholestane (LXIX) :

5,6β-Dibromo-5α-cholestane (LXV) (1.5 g) in benzene (20 ml) was reacted with morpholine (1.29 ml) over stirring for 1 hr. The reaction mixture was allowed to stand for 3 days at room temperature. The solvent was evaporated in vacuum and the residue was extracted with ether. The ethereal layer was washed several times with water and dried over anhydrous sodium sulphate. The oil obtained after evaporation of the solvent was chromatographed over silica gel (30 g).

5-Bromo-6β-morpholino-5α-cholestane (LXIX) :

Elution : pet. ether : ether (10:1), solvent of crystallization : acetone, Yield : (1.21 g), m.p. 160°C (positive Beilein test).

Analysis found : C, 69.65; H, 9.90

$C_{31}H_{53}NOBr$ requires : C, 69.66; H, 9.92%

IR : ν max 1185 – 1150 (C-N), 1075 (C-O), 670 cm^{-1} (C-Br).

^{1}H-NMR ($CDCl_3$) : δ 5.1 (dd, 1H, Jea = 4.4 Hz, Jee = 2.5 Hz, H-6α),

δ 2.4 – 2.1 (mc. 4H, $-CH_2-N-CH_2-$), 3.7 (mc, 4H, CH_2-O-CH_2-), 1.18 (C10-CH_3), 0.65 (C13-CH_3), 0.93 and 0.85 (side chain methyl protons).

^{13}C-NMR δ_C 36.0912 (C-1), 24.0132 (C-2), 27.9860 (C-3), 41.3411 (C-4), 86.8436 (C-5), 55.9863 (C-6), 36.7200 (C-7), 31.0254 (C-8), 45.3579 (C-9), 42.0904 (C-10), 21.3451 (C-11), 28.1456 (C-12), 42.5725 (C-13), 57.1285 (C-14), 23.7981 (C-15), 39.4598 (C-16), 56.5825 (C-17), 11.8895 (C-18), 19.6135 (C-19), 35.6624 (C-20), 18.6118 (C-21), 36.5126 (C-22), 23.7981 (C-23), 39.8126 (C-24), 28.1456 (C-25), 22.5455 (C-26), 22.7964 (C-27).

Mass : m/z (534/536, 1:1, M $\dot{+}$, absent), m/z 448/450, m/z 433/435, m/z 368.

Reaction of 5,6-dibromo-5α-cholestan-3-one (LXVI) with morpholine : 6β-Aminomorpholinocholest-4-en-3-one (LXX) :

5,6-Dibromo-5α-cholestan-3-one (LXVI) (1.5 g) in benzene (20 ml) reacted with morpholine (1.29 ml) over stirring for 1 hr. Reaction mixture was allowed to stand for 3 days at room temperature. The solvent was evaporated

in vacuum and the residue was extracted with ether. Ethereal layer was washed several times with water and dried over anhydrous sodium sulphate. The oil obtained after evaporation of the solvent was chromatographed over silica gel (30 g).

6β-Aminomorpholinocholest-4-en-3-one (LXX) :

Elution : pet. ether : ether (10:1), solvent of crystallization – acetone, Yield : (1.32 g), m.p. 160°C.

Analysis found : C, 69.65; H, 9.90

$C_{31}H_{53}NOBr$ requires : C, 69.66; H, 9.92%

IR : ν max 1690 (C=C-C=O), 1605 (C=C), 1185, 1120 cm^{-1} (C-H).

^{1}H-NMR ($CDCl_3$) : δ 6.16 (s, 1H, C4 [-olefinic), 5.2 (dd, 1H, Jea = 4.5 Hz, Jee = 2.5, H-6α), 1.15 (C10-CH_3), 0.70 (C13-CH_3), 0.95 and 0.90 (side chain methyl protons).

^{13}C-NMR : δ_C 36.0962 (C-1), 23.9285 (C-2), 199.4194 (C-3), 125.3974 (C-4), 161.0208 (C-5), 55.9276 (C-6), 36.0310 (C-7), 33.9346 (C-8), 45.7685 (C-9), 42.4992 (C-10), 20.8466 (C-11), 27.9662 (C-12), 39.7725 (C-13), 57.2135

(C-14), 23.7623 (C-15), 39.4223 (C-16), 56.1285 (C-17), 11.8585 (C-18), 17.4749 (C-19), 35.6352 (C-20), 18.6135 (C-21), 36.5215 (C-22), 23.9285 (C-23), 39.1045 (C-24), 28.3126 (C-25), 22.7720 (C-26), 22.5179 (C-27).

Mass : m/z 469 (M ⁺), m/z 454, m/z 383, m/z 356.

Reaction of 5, 6-dibromo-5α-cholestan-3-one (LXVI) with 2-aminopyrimidine: 6β-Aminopyrimidinocholest-4-en-3-one(LXXI):

5, 6-Dibromo-5α-cholestan-3-0ne (LXVI) (1.5 g) in benzene (20 ml), 2-aminopyrimidine (0.150 g) was added gradually over stirring for half an hour at room temperature. It was allowed to stand for 2 days at room temperature in vacuum and the residue was redissolved in ether. Ethereal layer was washed successively with water several times, very dilute hydrochloric acid solution, water, sodium bicarbonate solution (5%) and water and dried over anhydrous sodium sulphate.

6β-Aminopyrimidinocholest-4-en-3-one (LXXI) :

Solvent of crystallization : chloroform – methanol, Yield : (1.20 g), m.p. 180-181°C.

Analysis found : C, 77.97; H, 9.84

$C_{31}H_{47}N_3O$ requires : C, 77.98; H, 9.85%.

IR : ν max 3500-3450 (-NH), 1690 (C=O), 1605 (C=C), 1140 and 1080 cm^{-1} (C-N).

^{1}H-NMR ($CDCl_3$) : δ 8.35 (brs, 1H, exchangeable with deuterium, -NH), 5.4 (slightly resolved s, 1H, H-4-olefinic), 3.76 (mc, 1H, W½ = 7 Hz, H-6α), 1.1 (C10-CH_3), 0.7 (C13-CH_3), 0.90, 0.82 (side chain methyl protons).

^{13}C-NMR : δ_C 35.7909 (C-1), 23.3850 (C-2), 145.8456 (C-3), 42.6136 (C-4), 141.5633 (C-5), 56.7170 (C-6), 36.1949 (C-7), 33.3997 (C-8), 50.0908 (C-9), 43.4189 (C-10), 20.9747 (C-11), 28.0277 (C-12), 39.7725 (C-13), 56.1539 (C-14), 24.2790 (C-15), 39.5275 (C-16), 56.5228 (C-17), 11.8595 (C-18), 18.7269 (C-19), 36.1436 (C-20), 19.2644 (C-21), 35.8526 (C-22), 24.2136 (C-23), 39.1269 (C-24), 28.2183 (C-25), 22.5677 (C-26), 22.8235 (C-27).

Mass : m/z 477 ($M^{+\cdot}$, absent), m/z 383, m/z 382, m/z 368, m/z 270.

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CHAPTER - 4

Applications of X-ray in structure elucidation of steroids

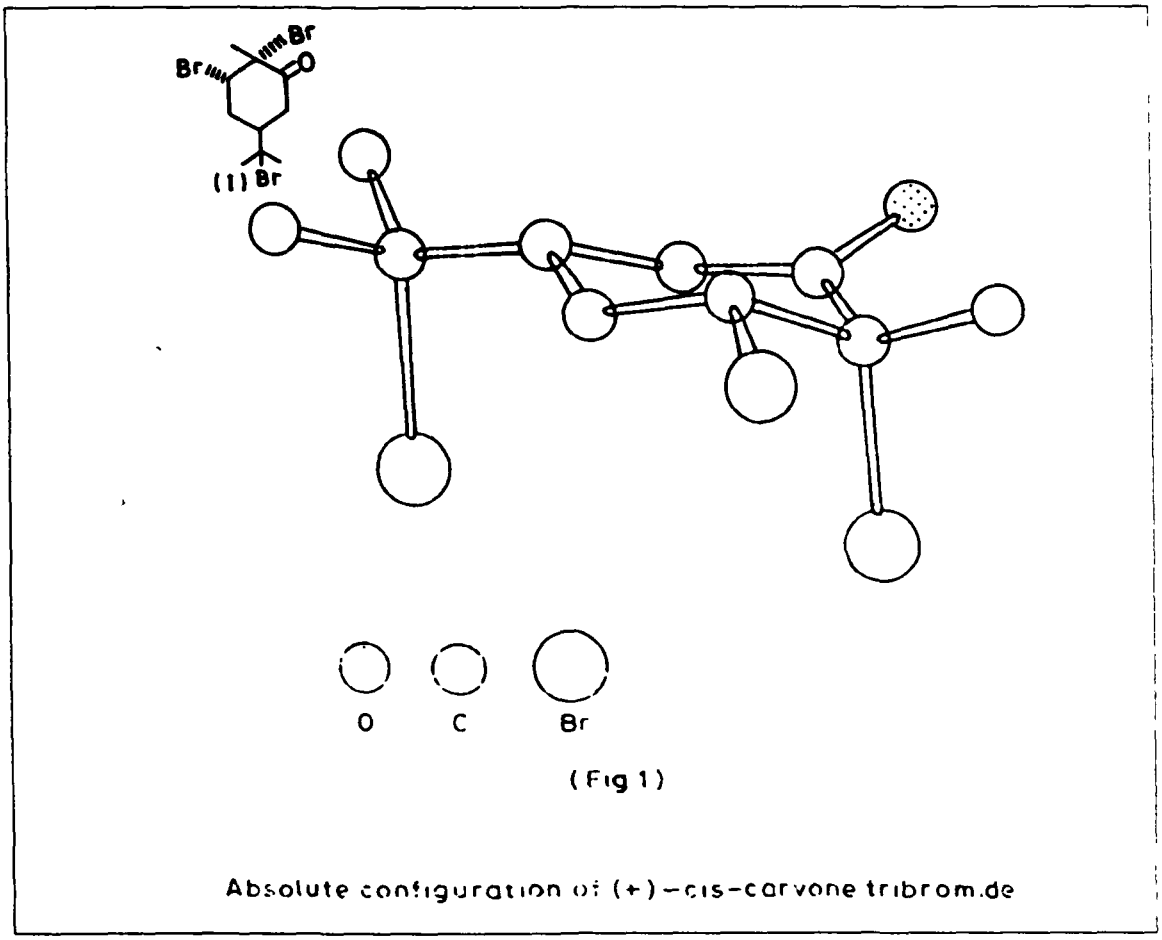
THEORETICAL

In recent years X-ray methods have been increasingly used for qualitative and quantitative analysis as well as for fundamental studies of the properties and structures of various classes of compounds, both organic and inorganic compounds. X-ray diffraction is the only convenient and hence widely used physical procedure for the complete determination of molecular structure.

X-rays were discovered by W. C. Rontgen in 1895 in the course of a systematic study to see if any radiation could be produced which would traverse matter opaque to ordinary light. He observed that when cathode rays produced in a highly evacuated discharge tube, strike a metal target (called an anticathode), new radiations were produced which he called X-ray for their nature were not known. The X-rays were found to be more penetrating than cathode rays. They affect a photographic plate, excite fluorescence in some compounds and produce ionization in gases. The radiations behave in noncorpuscular manner. They are not deflected by electrical or magnetic fields. They therefore do not consist of charged particles, they are electromagnetic waves analogous to light waves but of much shorter wave length. X-rays are diffusely scattered by all substances and are partially absorbed by matter of all kinds. The most useful method by which X-rays can

be used is X-ray diffraction method which involves the diffraction of X-rays from the plane of the crystals. The property of X-ray gives a analytical technique-X-ray crystallography which is now most useful method for structure elucidation of organic compounds with special reference to conformational analysis.

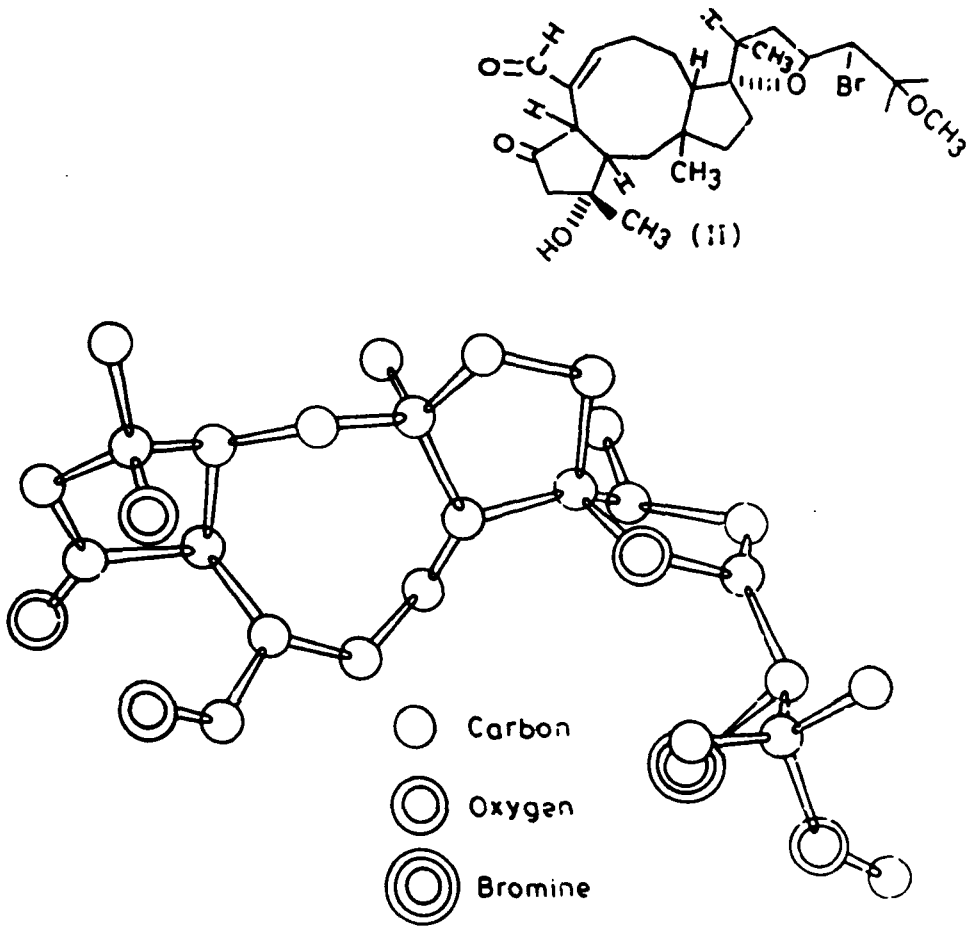
Richard W. Schevitz and co-workers[1] reported the structure of (+) –cis carvone tribromide (1) (Fig.1). The crystals of tribromide (1) are orthorhombic, a=18.40 ± 0.02, b=11.12 ± 0.02, c=6.32 ± 0.02 A°, space group $P2_12_12$, z = 4, molecules of $C_{10}H_{15}OBr_3$ per unit cell, d=1.98 g/ml (exp.)



(Fig 1)

Absolute configuration of (+)-cis-carvone tribromide

The structure of ophiobolin, a C25 terpenoid having a novel skeleton was studied by S. Tamura et al.[2]. The bromo methoxy derivative (II) (Fig.2) crystallized from ether, was found to be an orthorhombic system with space group $P2_12_12_1$ with unit cell dimensions a = 13.19, b = 22.27, c = 8.46 A° and z=4 (four molecule in a cell, d=1.40 /cm^3.

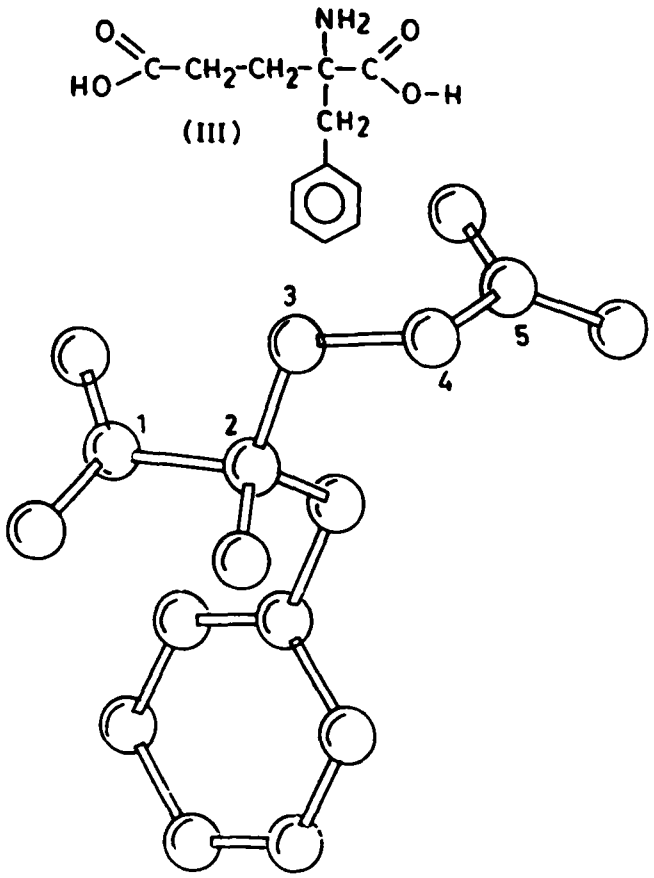


(II)

(Fig.2)

Molecular structure of ophiobolin methoxy bromide

X-ray crystallographic analysis, of D-(+)-2-benzyl glutamic acid (III) (Fig. 3a-c) was done by Tamaichi Ashida and co-workers[3]. Crystals are orthohombic, z = 4 (four molecules in unit cell), cell dimensions; a = 8.38; b=10.54; c = 17.68A°, space group; $P2_12_12_1$. density = 1.515 g cm^3 radiation used, CuKα.



(Fig 3a)

Absolute configuration of D-(+)-2-benzylglutamic acid

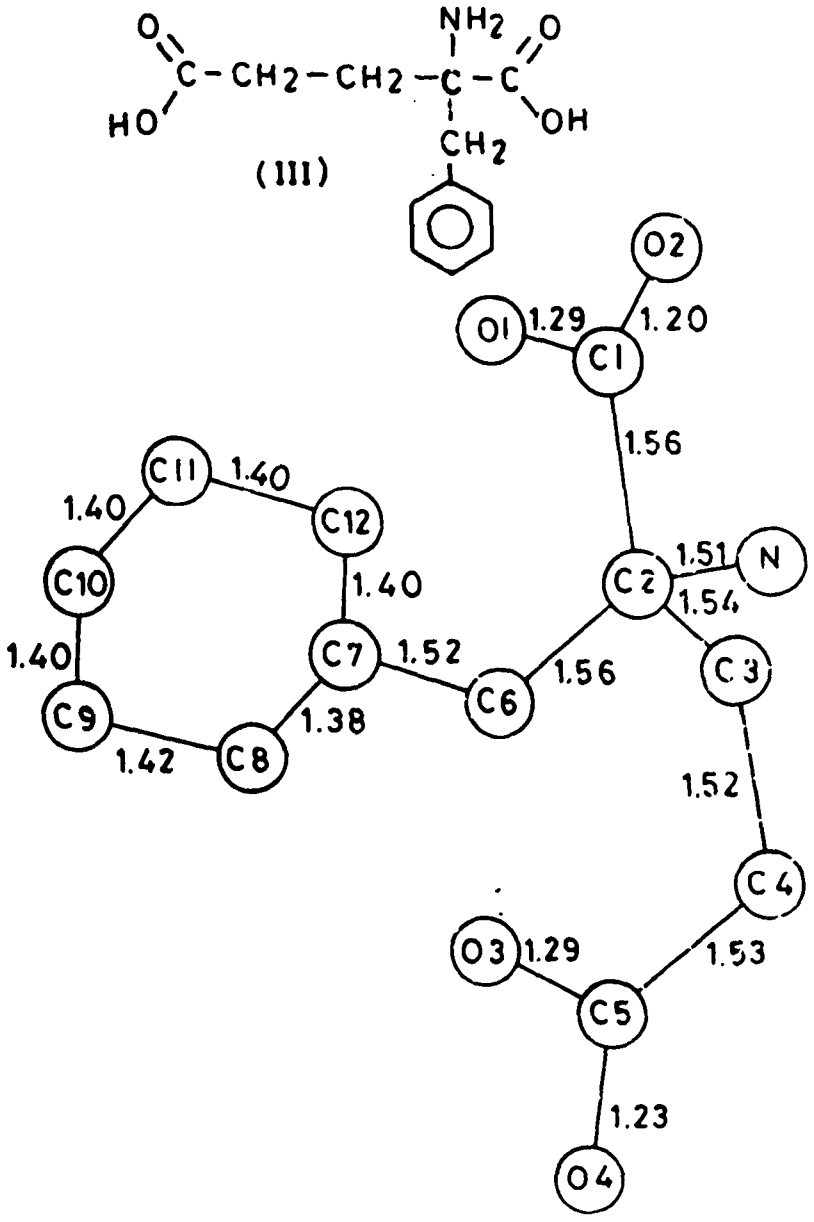
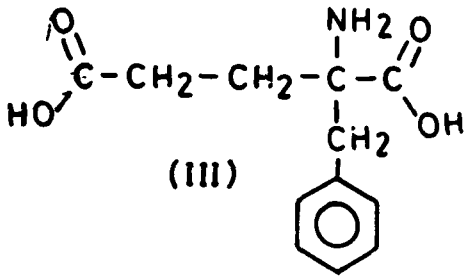


Fig. 3b : Bond Length in A° in
D–(+)–2–benzyl glutamic acid



(III)

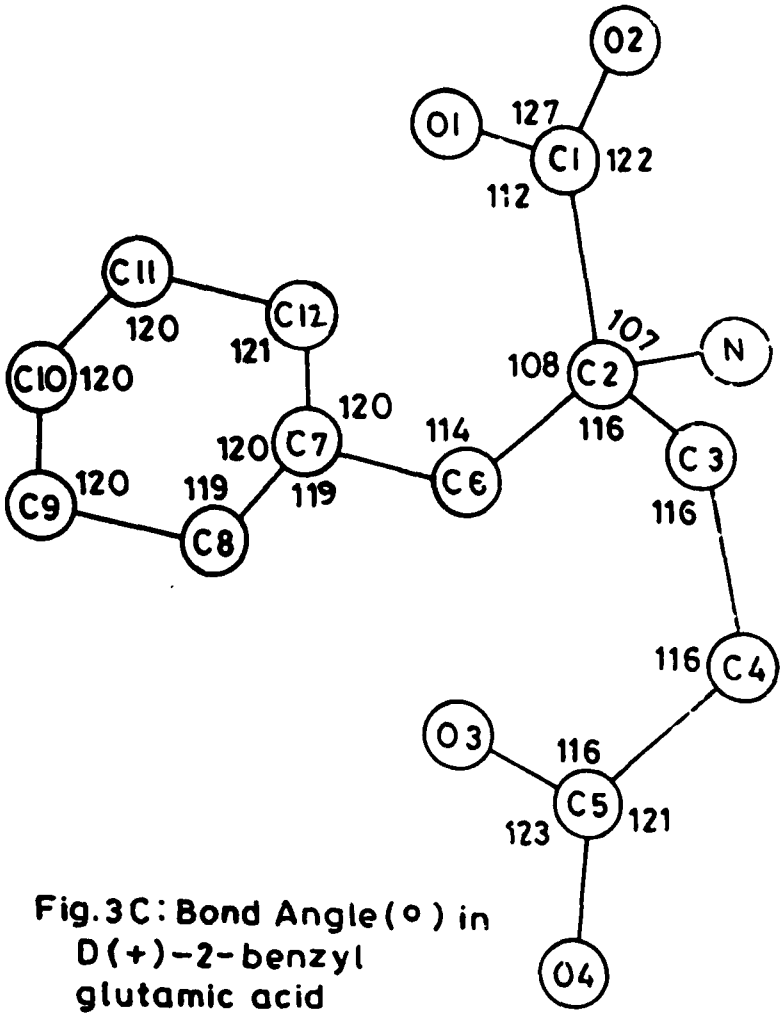
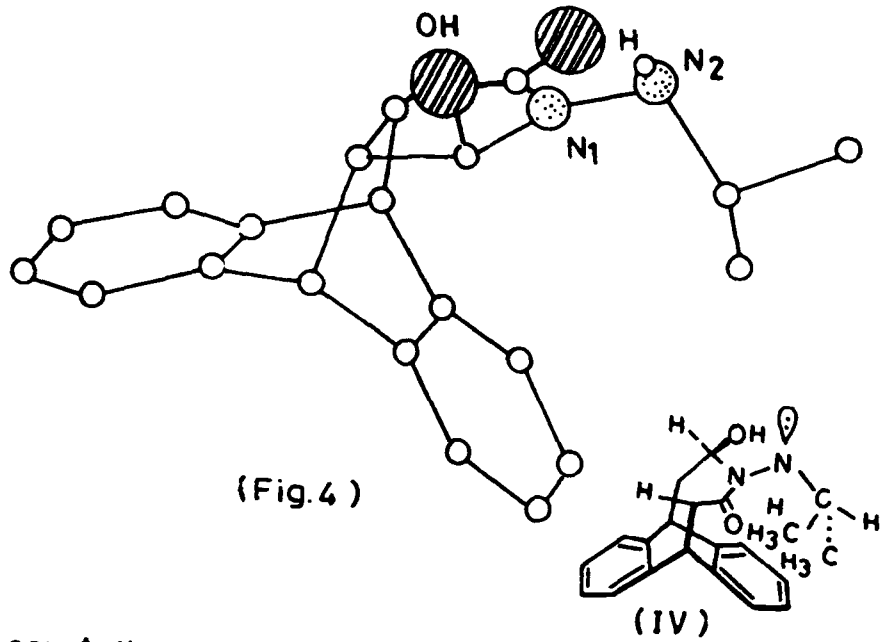


Fig.3C: Bond Angle(°) in D(+)-2-benzyl glutamic acid

The X-ray crystal structure analysis of N-(Isopropylamino)-3, 4-endo-(9′, 10′-dihydroanthracene 9′, 10′-diyl)-δ-exohydroxy-2-pyrrolidone (IV) (Fig. IV) was done by S. M. Verma and co-workers[4]. They reported different parameters : Crystal system monoclinic, space group $P2_1/C$, z = 4 (four molecule per unit cell), cell dimensions : a=11.553(2), b=17.045(3), c=10.975(2) A°, β=117.3(1); radiation used CuKα, λ=1.5418 A°, crystal size = 0.15x0.20x0.25 mm.

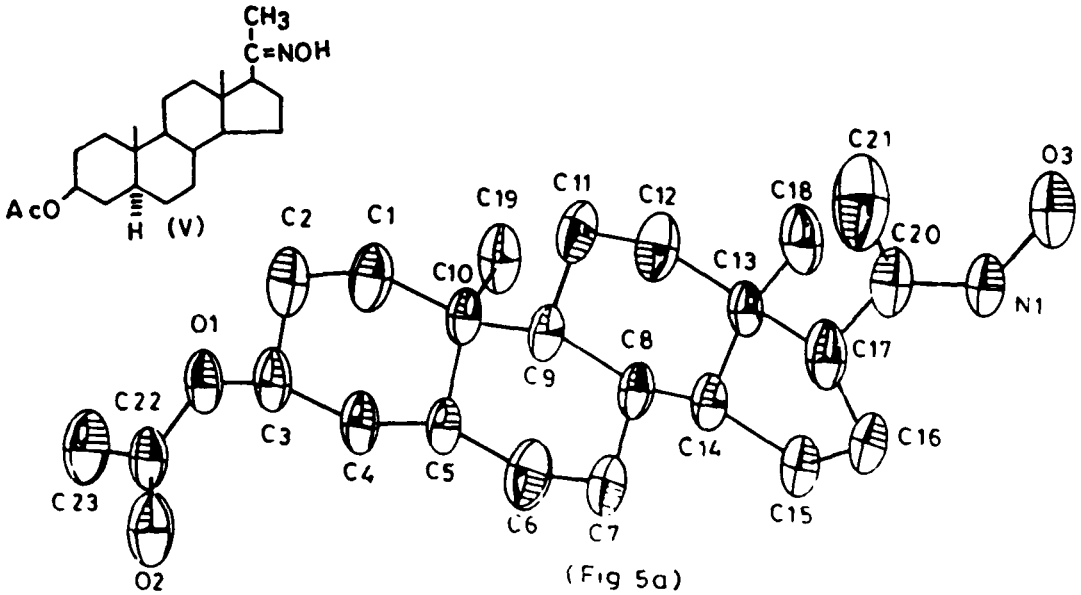


(Fig. 4)

(IV)

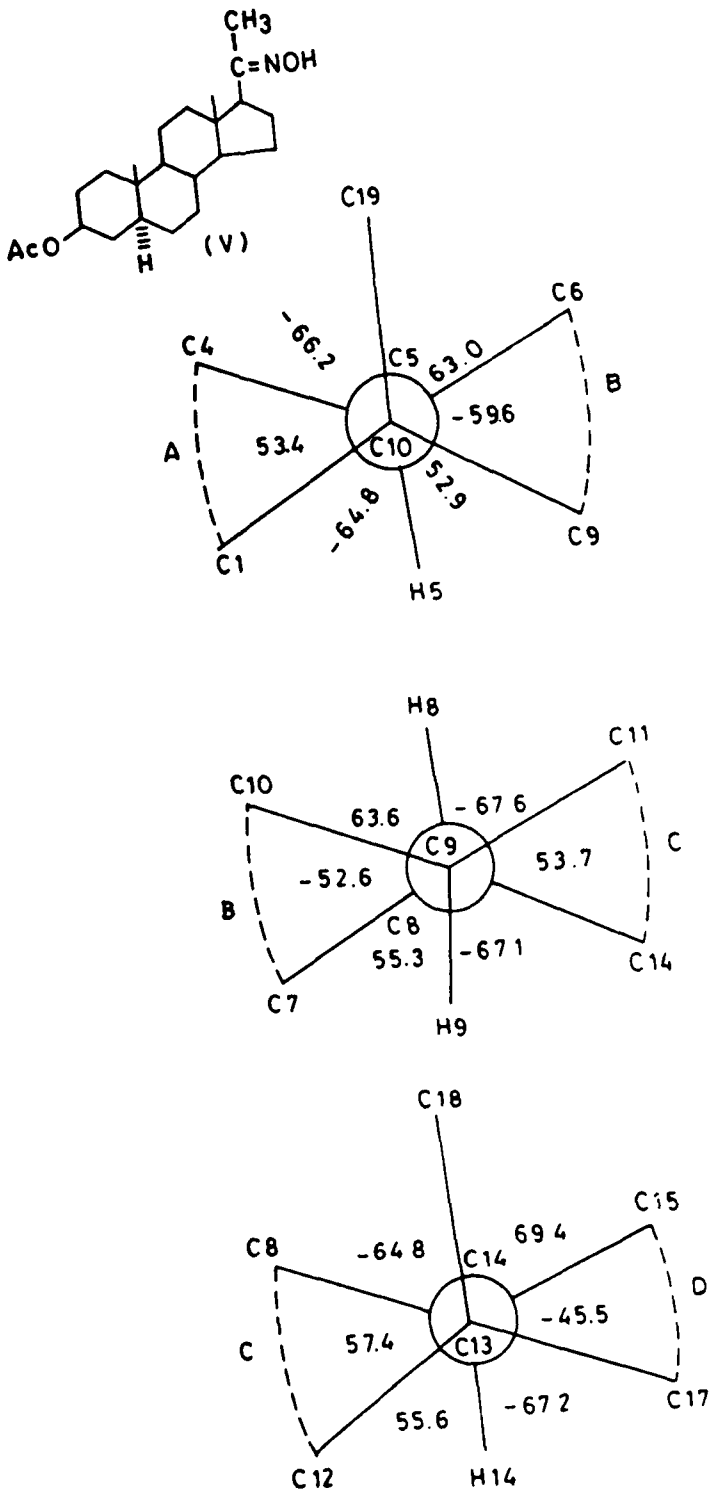
X-Ray crystallographic computer-generated perspective drawing of (IV)

Rajnikant and co-worker[5] studied the crystal structure of 3β-acetoxy-20-oximino-pergnane (V) (Fig. 5a,b) and reported the data related to this molecule : $C_{23}H_{37}NO_3$ (molecular weight = 375.5 amμ), a steroid crystallizes into orthorhombic, space group $P2_12_12_1$, volume = 2176A°^{3}. The unit cell parameters with estimated standard deviations (as given in parentheses) are : a = 11.609(3), b = 11.975(1), c = 15.653(4)A°, α = β = γ = 90°, z = 4 (four molecules per unit cell). The radiation used is CuKα λ = 1.5418A°.



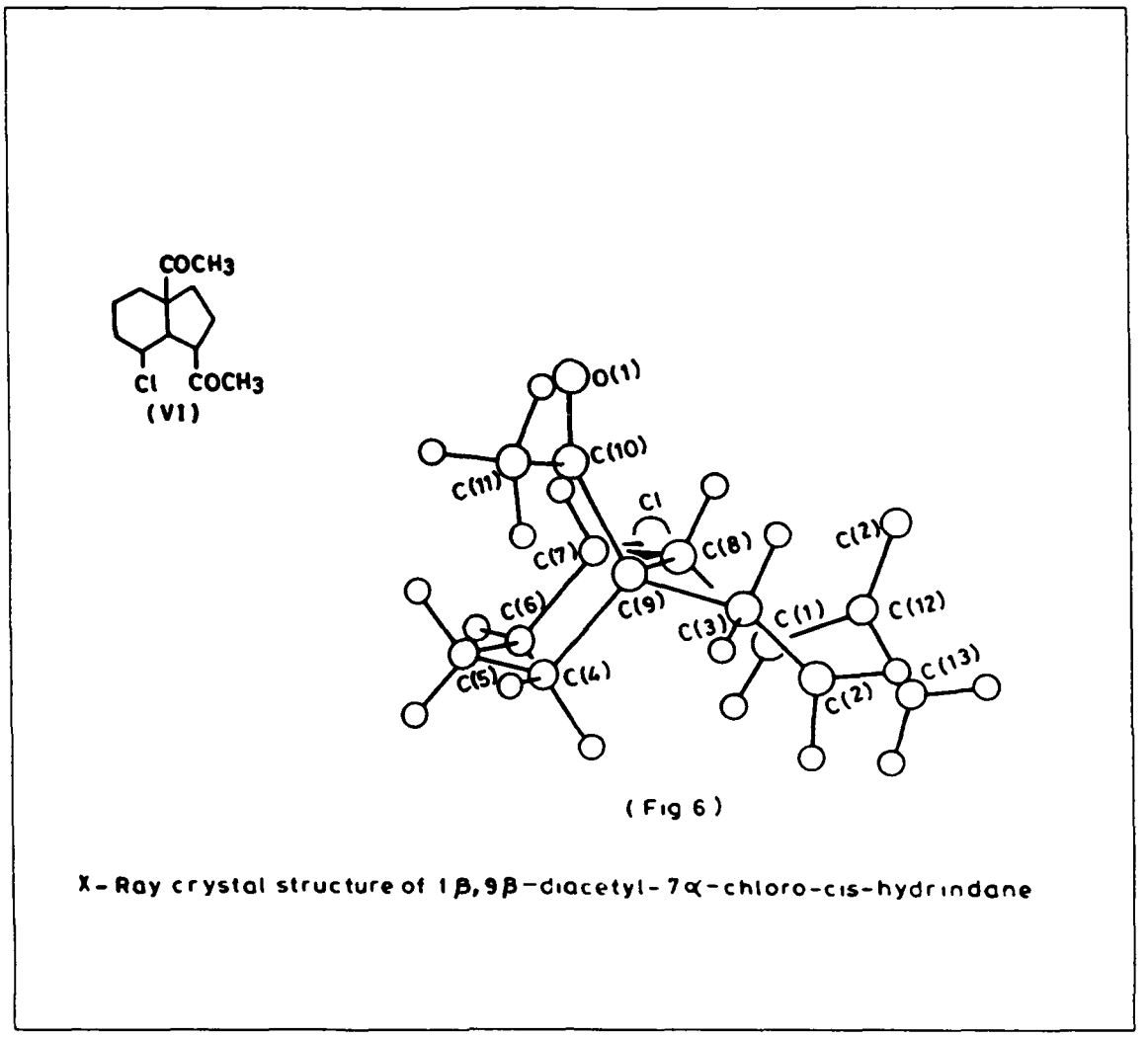
(Fig 5a)

General view of the molecule



(Fig 5b)
Newman projections along the bonds involved in ring fusion

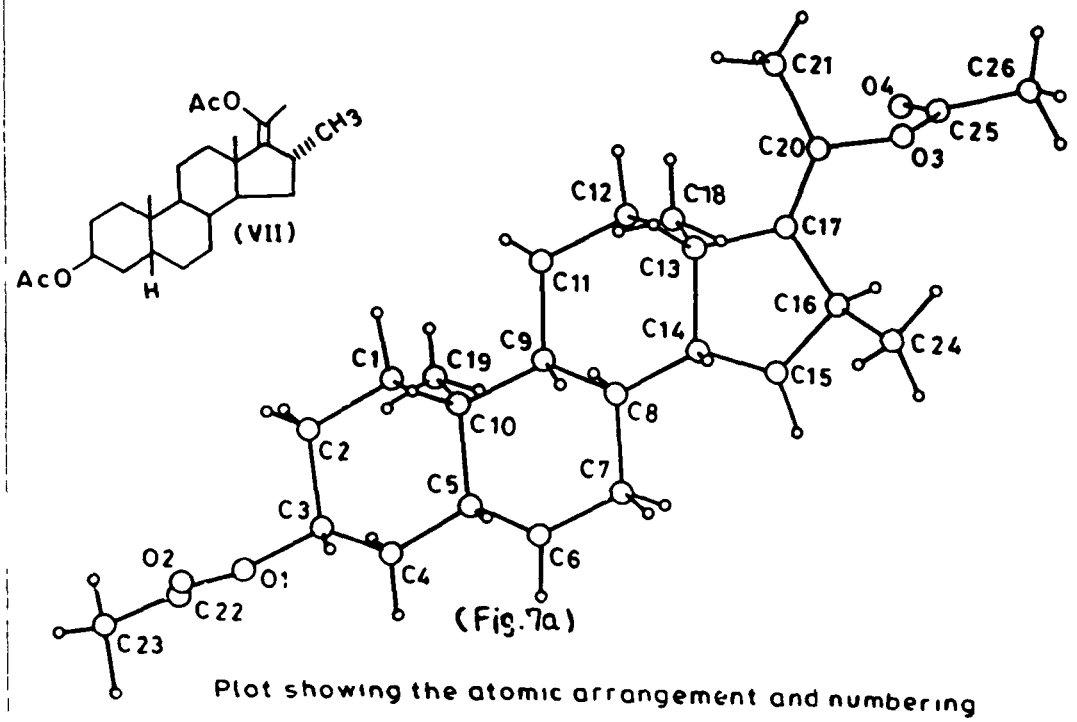
During the studies of Fridel Crafts acylation followed by hydrolysis of hydrindane J.W. Rasburn and co-workers[6] isolated 1β, 9β-diacetyl-7α-chloro-cis-hydrindane (VI) (Fig. **6**). They have also studied the X-ray crystal structure of the isolated compound and found the crystal data: ($C_{13}H_{19}ClO_2$, molecular weight = 242.7), monoclinic, space group $P2_1/c$, a=10.867 (1), b=10.334 (2), c=11.869 (1) A°, volume 1300.6 (3) $A^{3\circ}$, z=4 (four molecules in a cell). Mo-Kα radiation, λ=0.71073 A°.



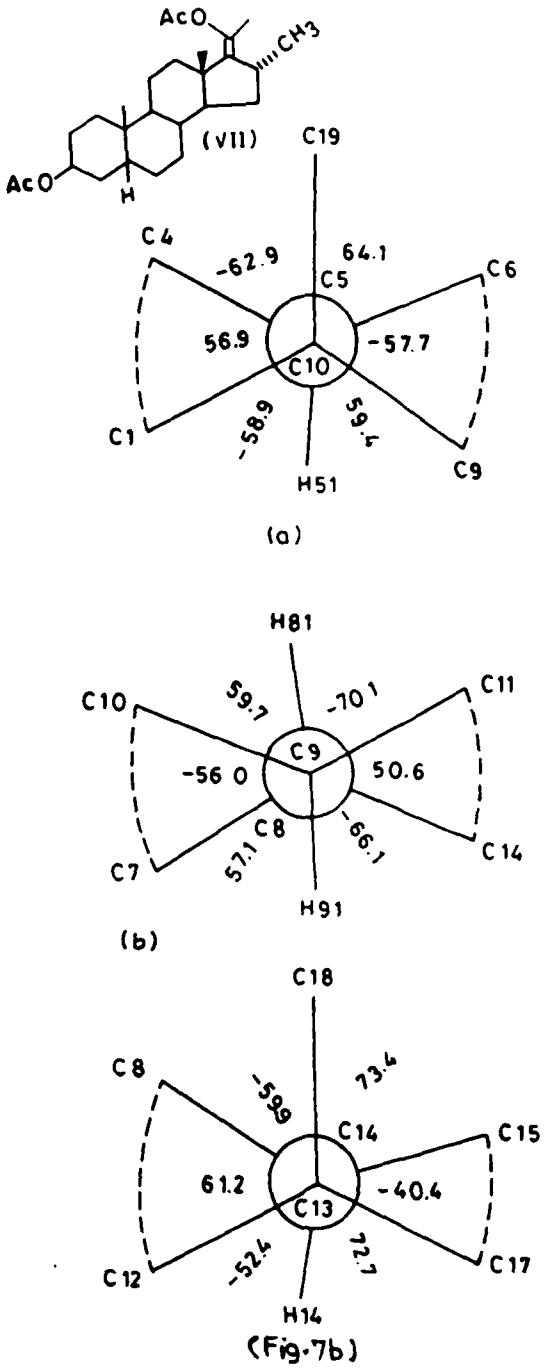
(Fig 6)

X-Ray crystal structure of 1β,9β-diacetyl-7α-chloro-cis-hydrindane

Rajnikant and co-workers[7] reported the results of X-ray analysis of 3β, 20-diacetoxy-16α-methyl-allopregn-17 (20)-ene (VII) (Fig. 7a,b). The X-ray data : ($C_{26}H_{40}O_4$, molecular weight = 416.6 g/mol). Reddish brown rectangular, crystal size : 0.22 x 0.38 x 0.46 mm, crystal system : orthorhombic, space group : $Pz_1z_1z_1$, cell dimensions : a=10.754 (2), b=11.190 (3), c=20.208 (1) A°, volume : 2429.7 $A^{\circ 3}$, z=4 (four molecules per unit cell).



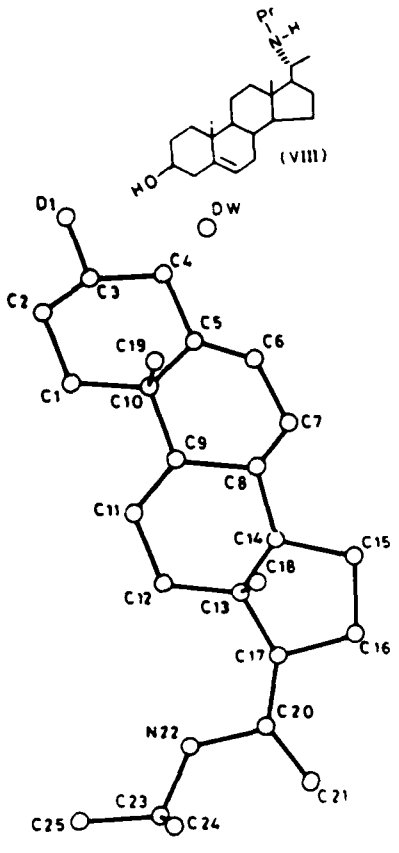
Plot showing the atomic arrangement and numbering



(Fig.-7b)

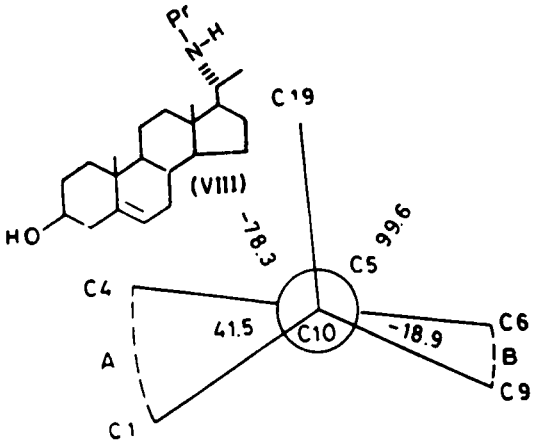
Newman projections along (A) c (5)-c (10), (B) c (8)-c (9) and (C) c (13)-c (14) (angle in °)

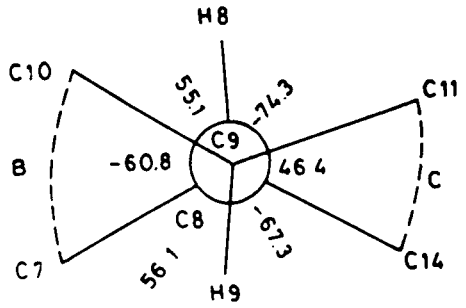
Vivek K. Gupta and coworkers[8] studied the crystal structure of 20α-propyl amino-3β-hydroxy-pregn-5-ene (VIII) (Fig. 8a-c) with water molecule – a steroid ($C_{24}H_{41}NO.H_2O$ molecular weight = 377.61 g/mol), the crystal structure has been determined by X-ray structure analysis. The compound crystallized as transparent rectangular, crystal size : 0.18 x 0.38 x 0.56 mm, in the monoclinic, space group $P2_1$ with cell parameters a = 11.901 (5), b = 8.098 (2), c = 12.398 (6) A°, β = 107.40 (2) °, volume = 1140.17A^{o3}, z = 2 (two molecules per unit cell), density (calculated) dc = 1.100 g/cm^3. The structure has been solved by direct methods.

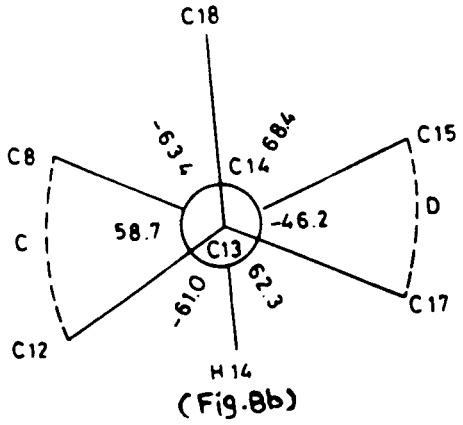


(Fig 8a)

General view of the molecule

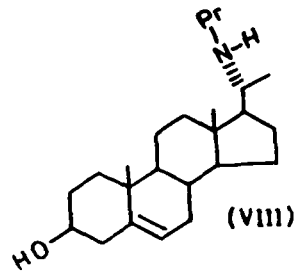






(Fig.8b)

Newman projections along (A)c(5)-c(10), (B)c(8)-c(9)and (C)c(13)-c(14)(angle in°)



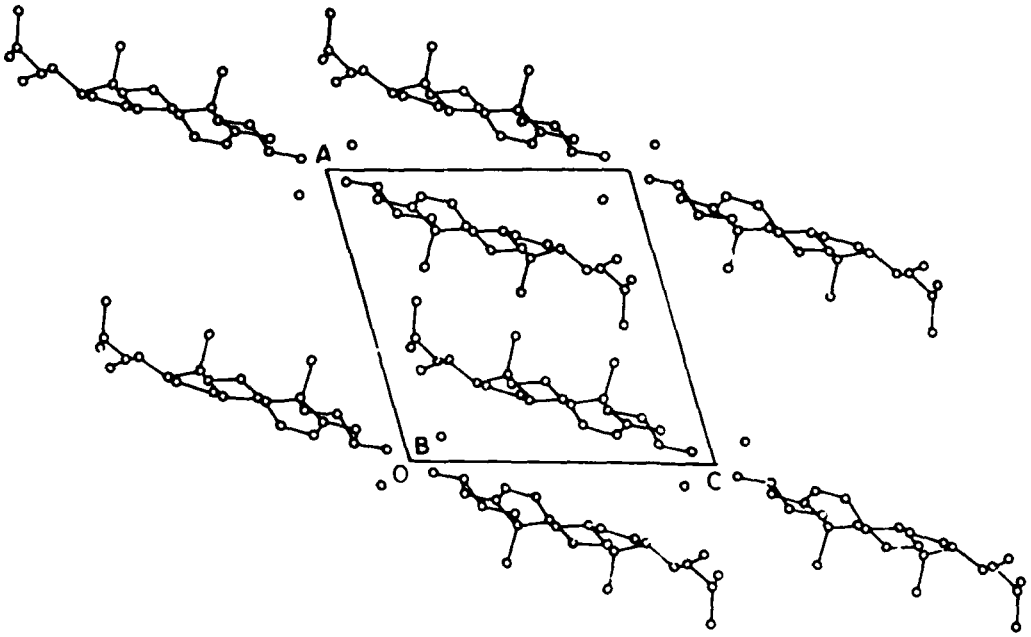


Fig 8C

Packing diagram viewed down the b-axis

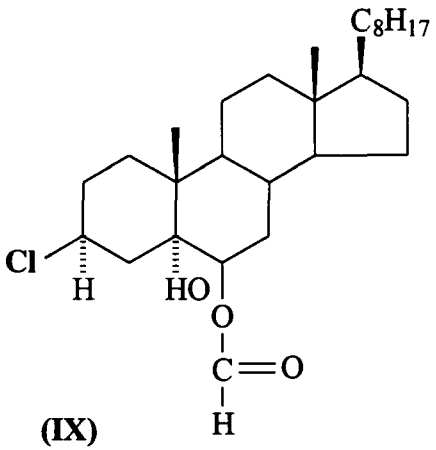
DISCUSSION

The first complete X-ray crystal structure analysis of a steroid (cholesteryl iodide) including determination of atomic coordinates was done 1945[9]. Since that time full crystal structure determination have been published for 234 steroids. A breakdown of these steroids according to class is presented in table below.

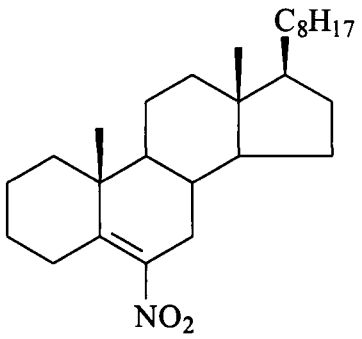
Class	Determined	Class	Determined
Estranes	37	Cholestanes	19
		Steroid alkaloids	11
Androstanes	75	Ergostanes	11
Pregnanes	59	Lanostanes	8
Cholanes	9	Cardanolides	4
		Bufanolides	1

These structure determinations provided a wealth of raw material concerning molecular conformation and intermolecular interactions. Previous work[10,11] from these laboratories described the crystal analysis of randomely chosen steroidal compounds such as 3β-chloro-6β-formyloxy-5α-cholestan-5-ol (IX) 6-nitrocholest-5-ene (X), 5α-cholestan-6-one (XI)and 3β-chloro-6-

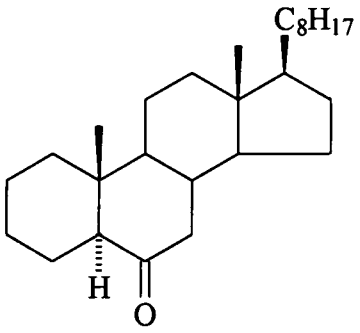
nitrocholest-5-ene (XII) and and useful information and data regarding the structure of these molecules (IX – XII) were obtained.



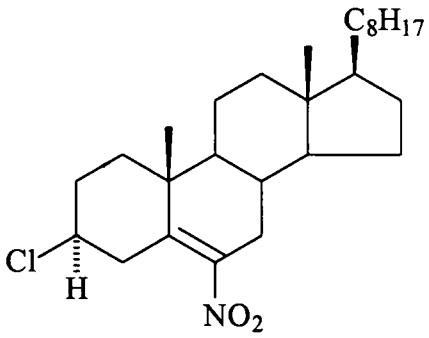
(IX)



(X)



(XI)



(XII)

X–Ray crystal analysis of 3β-chloro-6β-formyloxy-5α-cholestan-5-ol (IX) :

3β-Chloro-6β-formyloxy-5α-cholestan-5-ol (IX) with molecular composition $C_{28}H_{47}ClO_3$ (molecular weight, 467.11) gave[10] monoclinic crystals, with space group $P2_1$, a = 18.561 (2), b = 9.6233 (8), c = 7.8360 (1)

A°, volume = 1399.65 $A^{\circ 3}$, Z = 2(two molecule per unit cell), Dx = 1.133 g/cm^3. The structure has been determined by direct methods using SHELXS 86 (SHELDRICK, 1986)[12,13].

X – Rays analysis of 6-nitrocholest-5-ene (X)

X-Ray crystal structure analysis[11] of 6-nitrocholest-5-ene (X) ($C_{27}H_{45}NO_2$, molecular weight = 415) crystallized in the monoclinic form gave the results: space group $P2_1$, the unit cell parameters with estimated standard deviations (as given in parentheses), a = 12.143 (2), b = 10.853 (2), c = 19.747 (4) A°, Z =2 (two molecules per unit cell).

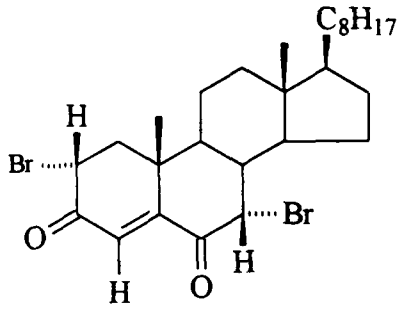
X-Ray structure analysis of 5α-cholestan-6-one (XI) :

The crystal structure of 5α-cholestan-6-one (XI) ($C_{27}H_{46}O$) has been studied by X-ray crystallographic analysis[11]. It crystallized into the monoclinic, space group was $P2_1$. The unit cell parameters with estimated standard deviations (as given in parentheses) are a = 10.575 (1), b = 7.698 (1), c = 15.284 (2) A°, α = 90°, β = 99.34 (I), γ = 90°. The molecular weight = 386.64 (amμ) volume (v): 1227.7 (3) $A^{\circ 3}$, Z = 2 (two molecules per unit cell).

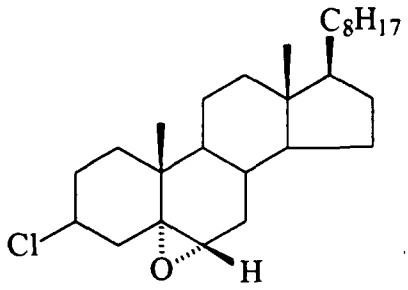
X-Ray structure analysis of 3β-chloro-6-nitrocholest-5-ene (XII):

Recently a paper entitled structure analysis of 3β-chloro-6-nitrocholest-5-ene (XII) was communicated by us in Molecular Materials (Russia - 1999)[14], in which the X-Ray structure analysis of (XII) has been taken up as a part of our crystallographic investigations on steroids. The compound (XII) ($C_{27}H_{44}NO_2Cl$, molecular weight = 450.10 amu) crystallized into orthorhombic, space group $P2_12_12_1$ with unit cell parameters, a = 7.207 (1), b = 11.292 (1), c = 32.373 (5) A°, $\alpha = \beta = \gamma = 90°$ and volume (V) of the unit cell having 4 molecules per unit cell (Z=4) was found 2634.56 $A°^3$.

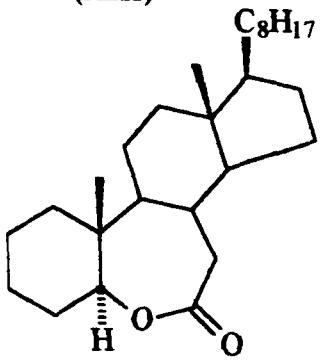
In continuation to our work done in X-ray crystallography, the X-ray crystal structure analysis of 2α, 7α-bromocholest-4-ene-3, 6-dione (XIII), 3β-chloro-5, 6β-epoxy-5α-cholestane (XIV) and 6-oxa-B-homo-5α-cholestan-7-one (XV) and 5α-cholestane-3, 6-dione (XVI) were thoroughly studied and different parameter of the compounds were given.



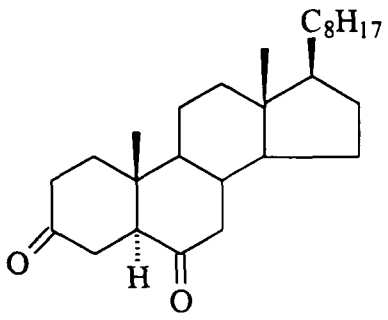
(XIII)



(XIV)



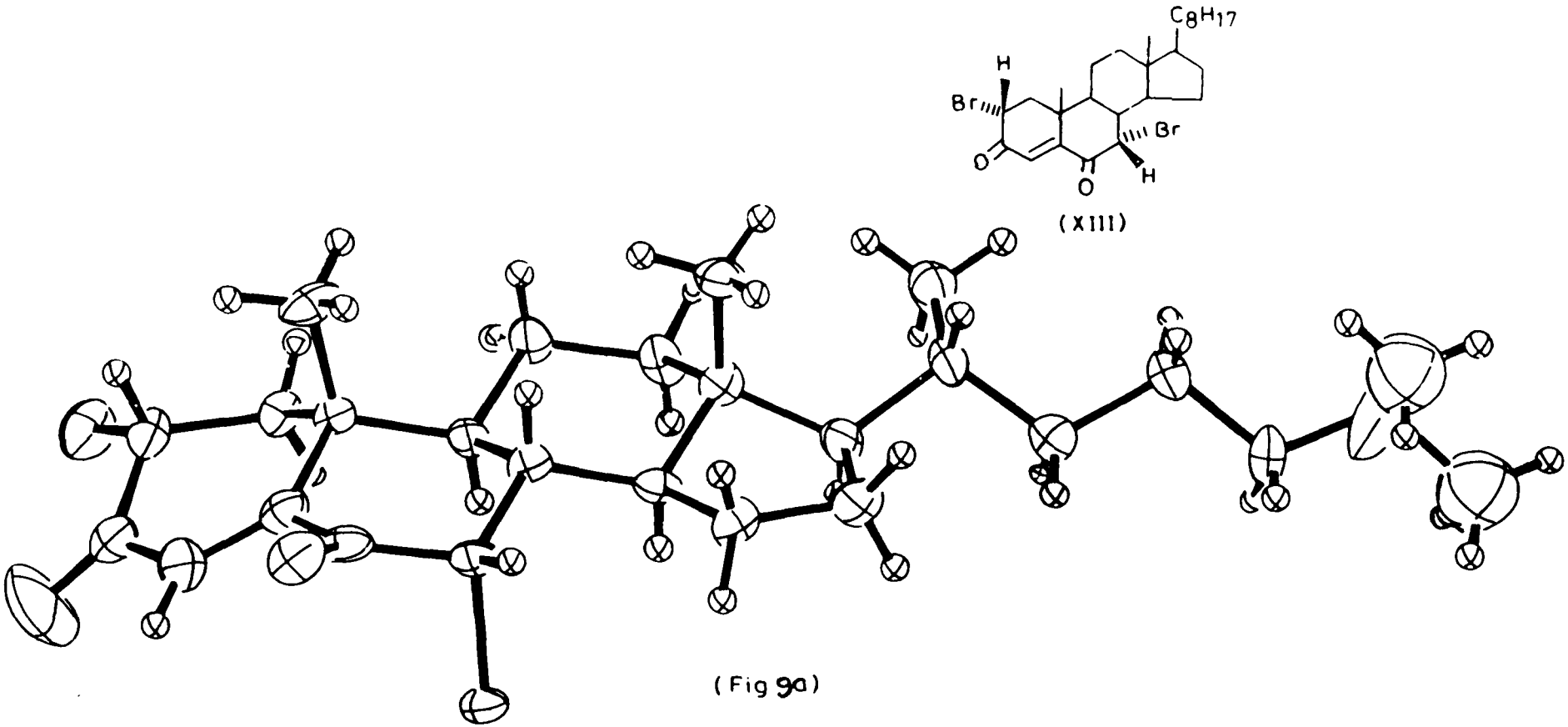
(XV)



(XVI)

X-Ray analysis of 2α-7α-dibromocholest-4-ene-3, 6-dion (XIII)

The X-ray analysis of 2α- 7α-dibromo cholest-4-ene-3, 6-dione[15] (XIII)(Fig. 9a,b) ($C_{27}H_{40}O_2Br_2$, molecular weight = 556.62) was done with crystal morphology results: colourless, prism, crystal dimensions (mm) 0.50 x 0.50 x 0.50, crystal system; monoclinic, Lattice dimensions; a = 11.585 (2), b = 7.648 (2), c = 15.323 (1) A°, β = 93.803 (9) A°; volume, 1354.6 (4) $A^{\circ 3}$, space group, $P2_1$; Z = 2 (two molecules per unit cell), Density (Dc); 1.364



(XIII)

(Fig 9a)

General view of the molecule

g/cm^3; Radiation, Cukα λ = 1.54178 A°; temperature, 23 °C, structure solutions; direct methods.

Other important parameters obtained by X-ray crystallography of 2α, 7α-dibromo-cholest-4-ene-3, 6-dione (XIII) are bond lengths and bond angles of different bonds involving hydrogen and non hydrogen atoms in the molecule. The values of these parameters are given in tables 1-4.

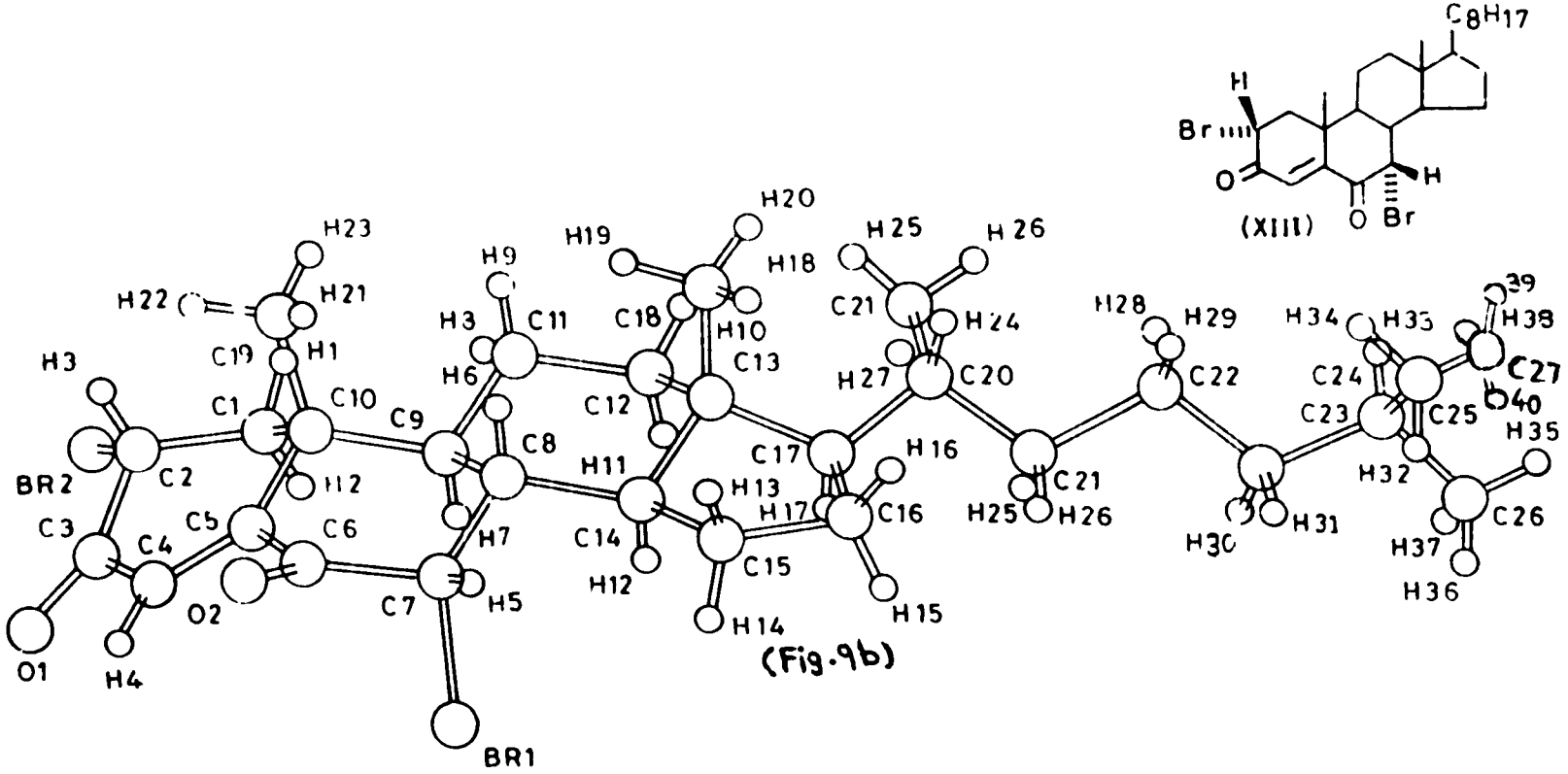
Table - 1

Intramolecular distance involving hydrogen atoms

Atom	Atom	Distance	Atom	Atom	Distance
C_1	H_1	0.955	C_{19}	H_{21}	0.951
C_1	H_2	0.947	C_{19}	H_{22}	0.949
C_2	H_3	0.948	C_{19}	H_{23}	0.955
C_4	H_4	0.957	C_{20}	H_{24}	0.951
C_7	H_5	0.949	C_{21}	H_{25}	0.945
C_8	H_6	0.948	C_{21}	H_{26}	0.951
C_9	H_7	0.950	C_{21}	H_{27}	0.952

C_{11}	H_8	0.951	C_{22}	H_{28}	0.952
C_{11}	H_9	0.948	C_{22}	H_{29}	0.945
C_{12}	H_{10}	0.949	C_{23}	H_{30}	0.950
C_{12}	H_{11}	0.952	C_{23}	H_{31}	0.950
C_{14}	H_{12}	0.953	C_{24}	H_{32}	0.950
C_{15}	H_{13}	0.948	C_{24}	H_{33}	0.950
C_{15}	H_{14}	0.949	C_{25}	H_{34}	0.950
C_{16}	H_{15}	0.950	C_{26}	H_{35}	0.950
C_{16}	H_{16}	0.948	C_{26}	H_{36}	0.950
C_{17}	H_{17}	0.948	C_{26}	H_{37}	0.950
C_{18}	H_{18}	0.951	C_{27}	H_{38}	0.949
C_{18}	H_{19}	0.947	C_{27}	H_{39}	0.926
C_{18}	H_{20}	0.950	C_{27}	H_{40}	0.974

Distances are in angstroms, Estimated standard deviations in the least significant figure are given in parentheses.



(Fig.9b)

Plot showing atomic arrangement and numbering

Table - 2

Intramolecular bond angles involving the hydrogen atoms

Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle
C2	C1	H1	108.11	H18	C18	H20	109.79
C2	C1	H2	108.76	H9	C18	H20	109.43
C10	C1	H1	108.64	H18	C18	H19	109.69
C10	C1	H2	109.07	C10	C19	H21	109.77
H1	C1	H2	109.07	C10	C19	H22	109.92
BR2	C2	H3	107.62	C10	C19	H23	109.64
C1	C2	H3	107.66	H21	C19	H22	109.45
C3	C2	H3	107.42	H21	C19	H23	108.93
C3	C4	H4	118.78	H22	C19	H23	109.11
C5	C4	H4	118.67	C17	C20	H24	109.15
BR1	C7	H5	110.01	C22	C20	H24	108.86
C6	C7	H5	110.45	C21	C20	H24	109.02
C8	C7	H5	110.24	C20	C21	H25	109.58
C7	C8	H6	107.13	C20	C21	H26	109.28
C9	C8	H6	107.17	C20	C21	H27	109.15
C14	C8	H6	107.17	C25	C21	H26	109.85

C8	C9	H7	106.27	C25	C21	H27	109.73
C10	C9	H7	106.39	H26	C21	H27	109.24
C11	C9	H7	106.43	C20	C22	H28	107.73
C9	C11	H8	108.17	C20	C22	H29	108.23
C9	C11	H9	108.41	C23	C22	H28	108.09
C12	C11	H8	108.03	H28	C22	H29	108.51
C12	C11	H9	108.12	H28	C22	H29	109.77
H8	C11	H9	109.60	C22	C23	H30	108.98
C11	C12	H10	108.94	C22	C23	H31	109.26
C11	C12	H11	108.80	C24	C23	H30	109.05
C13	C12	H10	108.70	C24	C23	H31	109.05
C13	C12	H11	108.64	H30	C23	H31	109.46
H10	C12	H11	109.39	C23	C24	H32	106.97
C8	C14	H12	106.39	C23	C24	H33	106.97
C13	C14	H12	106.18	C25	C24	H32	106.97
C15	C14	H12	106.04	C25	C24	H33	106.97
C14	C15	H13	110.86	C25	C24	H32	106.97
C14	C15	H14	110.99	C25	C24	H33	106.97
C16	C15	H13	111.30	H32	C24	H33	109.46

C16	C15	H14	111.27	C24	C25	H34	112.19
H13	C15	H14	109.65	C26	C25	H34	112.19
C15	C16	H15	110.10	C27	C25	H34	113.17
C15	C16	H16	110.13	H35	C26	H37	109.47
C17	C16	H15	109.85	H36	C26	H37	109.47
C17	C16	H16	110.05	C25	C27	H38	109.41
H15	C16	H16	109.67	C25	C27	H39	110.81
C13	C17	H17	107.46	C25	C27	H40	107.84
C16	C17	H17	107.40	H38	C27	H40	111.68
C20	C17	H17	107.65	H38	C27	H39	107.57
C13	C18	H18	109.29	H39	C27	H40	109.40
C13	C18	H19	109.47				
H13	C18	H20	109.16				

Angles are in degrees. Estimated standard deviations in the least significant figure are given in parentheses.

Table – 3

Intramolecular distances involving the non-hydrogen atoms

Atom	Atom	Distance	Atom	Atom	Distance
BR1	C7	1.977 (9)	C10	C19	1.53 (1)
BR1	C2	1.94 (1)	C11	C12	1.52 (1)
O1	C3	1.22 (1)	C12	C13	1.51 (1)
O2	C6	1.21 (1)	C13	C14	1.55 (1)
C1	C2	1.53 (1)	C13	C17	1.56 (1)
C2	C10	1.54 (1)	C13	C18	1.54 (1)
C2	C3	1.51 (2)	C14	C15	1.53 (1)
C3	C4	1.48 (2)	C15	C16	1.56 (1)
C4	C5	1.37 (1)	C16	C17	1.54 (1)
C5	C6	1.47 (1)	C17	C20	1.55 (1)
C5	C10	1.55 (1)	C20	C21	1.54 (1)
C6	C7	1.51 (1)	C20	C22	1.53 (1)
C7	C8	1.54 (1)	C22	C23	1.51 (2)
C8	C9	1.52 (1)	C23	C24	1.50 (1)
C8	C14	1.52 (1)	C24	C25	1.59 (2)
C9	C10	1.56 (1)	C25	C26	1.49 (2)
C9	C11	1.54 (1)	C25	C27	1.50 (2)

Distance are in angstroms. Estimated standard deviation in the least significant figure are given parentheses.

Table - 4

Intramolecular bond angles involving the non-hydrogen atoms

Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle
BR2	C2	C1	110.1 (7)	C5	C10	C19	106.6 (7)
BR2	C2	C3	110.7 (7)				
O1	C3	C2	125.0 (1)	C9	C10	C19	112.7 (7)
O1	C3	C4	120.0 (1)	C9	C11	C12	114.5 (8)
BR1	C7	C6	101.9 (6)	C11	C12	C13	112.3 (8)
BR1	C7	C8	111.9 (6)	C12	C13	C14	107.2 (7)
O2	C6	C5	122.7 (9)	C12	C13	C17	116.4 (8)
O2	C6	C7	121.2 (9)	C12	C13	C18	108.7 (8)
C2	C1	C10	112.9 (8)	C14	C13	C17	98.4 (7)
C1	C2	C3	113.2 (9)	C14	C13	C18	112.9 (8)
C2	C3	C4	114.6 (9)	C17	C13	C18	112.9 (7)
C3	C4	C5	123 (1)	C8	C14	C13	113.7 (7)
C4	C5	C6	118.0 (1)	C8	C14	C15	118.7 (8)

C4	C5	C10	124.0 (1)	C13	C14	C15	105.0 (7)
C6	C5	C10	118.2 (8)	C14	C15	C16	102.6 (8)
C5	C6	C7	116.1 (8)	C15	C16	C17	107.0 (8)
C6	C7	C8	112.1 (7)	C13	C17	C16	104.1 (8)
C7	C8	C9	113.3 (7)	C13	C17	C20	119.5 (8)
C7	C8	C14	112.3 (7)	C16	C17	C20	110.2 (8)
C9	C8	C14	109.1 (7)	C17	C20	C22	108.5 (8)
C8	C9	C10	114.6 (7)	C17	C20	C21	112.0 (8)
C8	C9	C11	110.4 (7)	C22	C20	C21	109.2 (9)
C10	C9	C11	112.2 (7)	C20	C22	C23	114.5 (8)
C1	C10	C5	110.1 (7)	C20	C22	C24	111.0 (4)
C1	C10	C9	108.9 (7)	C23	C24	C25	119.2 (9)
C1	C10	C19	110.8 (8)	C24	C25	C26	95.0 (9)
C5	C10	C9	107.7 (8)	C24	C25	C27	116.3 (7)
				C26	C25	C27	106.3 (7)

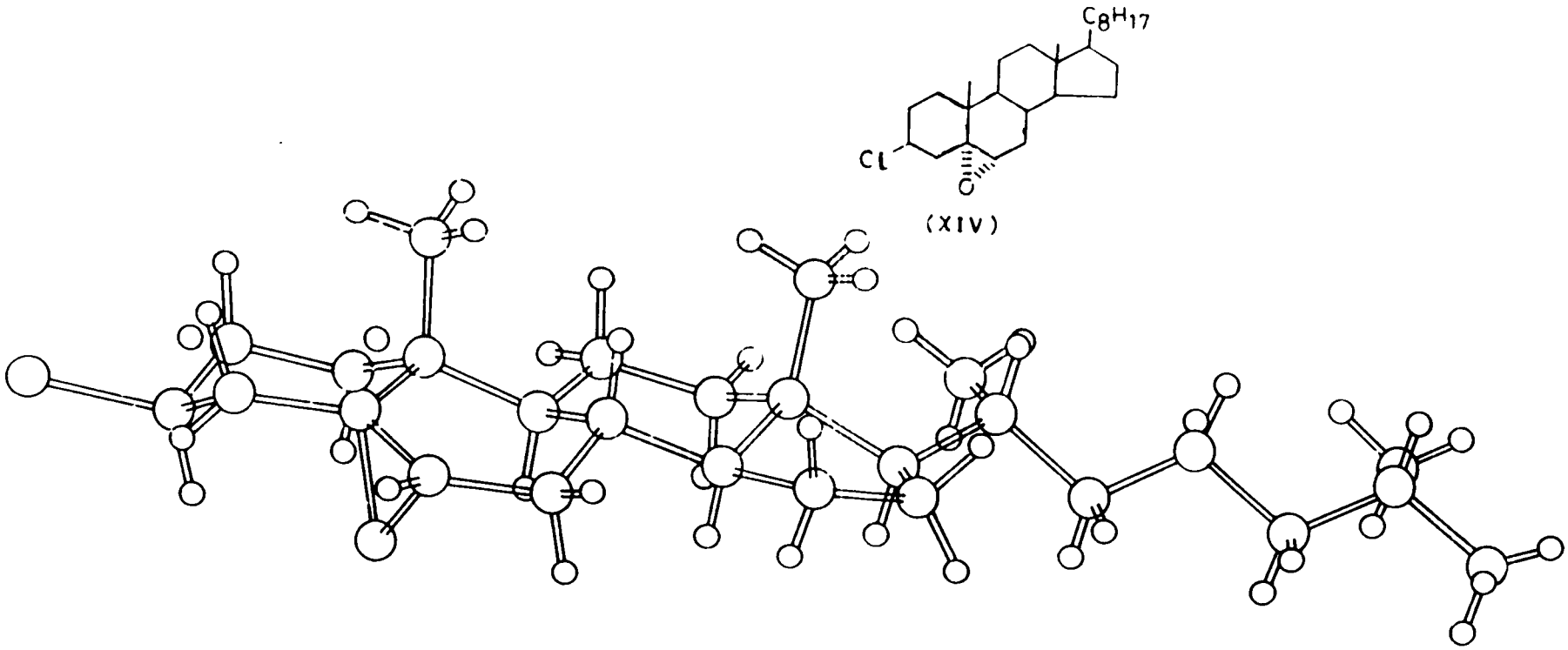
Angles are in degrees. Estimated standard deviations in the least significant figure are given in parentheses.

In the structure of 2α, 7α-dibromocholest-4-ene3,6-dione (XIII) the part of the molecule from C2 to C7 tending to lie in the same plan due to sp^2

nature of carbons involved causing significant change in the conformational shape of rings A and B. It is interesting to note that bromine attached to C2 being equatorial is lying very close to the plane of the carbonyl group while bromine being axial at C7 is moving away from carbonyl plane.

X-Ray analysis of 3β-chloro-5, 6α-epoxy-5α-cholestane (XIV)

X-Ray crystallography of 3β-chloro-5, 6α-epoxy-5α-cholestane (XIV)[16] (Fig.10 a, b) was done with the aim to know the conformational changes occurring in the molecule during the formation of epoxide from 3β-chlorocholest-5-ene (XIX) on treatment with metachloroperbenzoic acid. It has been found that the conformational changes in rings A and B particularly due to the formation of epoxide ring has occurred. The various parameters obtained during the X-ray crystallographic study : molecular formula; $C_{27}H_{45}ClO$, molecular weight; 421.10, crystal morphology; colourless, plate, crystal dimension (mm); 0.20 x 0.40 x 0.20, crystal system; orthorhombic, Lattice parameters; a = 22.80 (4), b = 7.671 (3), c=29.657(5) A°, y = 5012 (2) $A°^3$, space group; $P2_12_12_1$, Z = 8 (eight molecules in per unit cell), Density (Dc) 1.116 g/cm^3, radiation; wkα; λ=1.54178 A°, temperature; 23°C, structure solution; direct methods.



(XIV)

(Fig.10a)

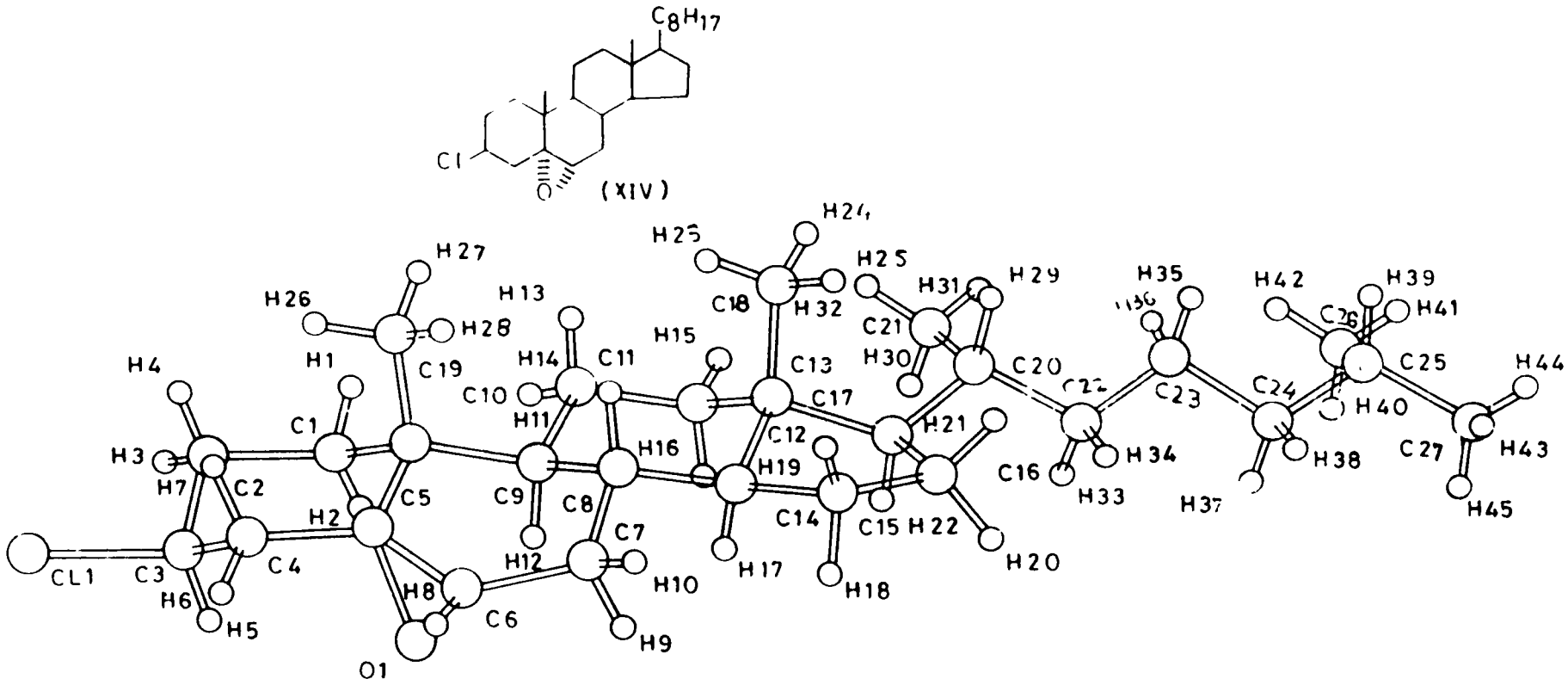
General view of the molecule

The most important data obtained by X-ray crystallography are bond length, bond angle and different structural and conformational shape of the molecule.

Table - 1

Intramolecular distances involving the hydrogen atoms

Atom	Atom	Distance	Atom	Atom	Distance
C1	H1	1.051	C18	H24	0.955
C1	H2	0.913	C18	H25	1.020
C2	H3	1.006	C19	H26	0.966
C2	H4	0.955	C19	H27	0.948
C3	H5	1.045	C19	H28	1.049
C4	H6	1.012	C20	H29	1.027
C4	H7	1.062	C21	H30	0.984
C6	H8	1.074	C21	H31	0.991
C7	H9	1.070	C21	H32	0.964
C7	H10	0.921	C22	H33	1.027
C8	H11	1.116	C22	H34	0.889
C9	H12	1.016	C23	H35	0.989
C11	H13	0.924	C23	H36	0.969



(Fig.10b)

Plot showing atomic arrangement and numbering

C11	H14	0.996	C24	H37	0.983
C12	H15	0.951	C24	H38	0.975
C12	H16	1.021	C25	H39	0.941
C14	H17	0.983	C26	H40	0.975
C15	C18	1.024	C26	H41	0.987
C15	H19	0.932	C26	H42	0.979
C16	H20	1.045	C27	H43	1.010
C16	H21	1.001	C27	H44	0.886
C17	H22	1.028	C27	H45	0.924
C18	H23	0.939			

Distances are in angstroms. Estimated standard deviations in the least significant figure are given in parentheses.

Table - 2

Intramolecular bond angles involving the hydrogen atoms

Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle
C2	C1	H1	109.86	C13	C17	H22	107.03
C2	C1	H2	116.98	C16	C17	H22	108.17

C10	C1	H1	104.64	C20	C17	H22	105.90
C10	C1	H2	106.29	C13	C18	H23	114.60
H1	C1	H2	104.11	C13	C18	H24	112.54
C1	C2	H3	107.85	C13	C18	H25	110.74
C1	C2	H4	113.38	H23	C18	H24	109.96
C3	C2	H3	113.12	H23	C18	H25	104.66
C3	C2	H4	109.11	C10	C19	H26	112.93
H3	C2	H4	104.48	C10	C19	H27	120.06
CL1	C3	H5	112.11	C10	C19	H28	110.71
C2	C3	H5	105.84	H26	C19	H27	108.35
C4	C3	H5	111.64	H26	C19	H28	100.66
C3	C4	H6	111.96	H27	C19	H28	101.87
C3	C4	H7	107.42	C17	C20	H29	111.77
C5	C4	H6	119.03	C21	C20	H29	104.12
C5	C4	H7	112.45	C22	C20	H29	108.03
H6	C4	H7	96.79	C20	C21	H30	109.74
O1	C6	H8	118.24	C20	C21	H31	115.74
C5	C6	H8	120.10	C20	C21	H32	116.02
C7	C6	H8	111.55	H30	C21	H31	103.51

C6	C7	H9	106.35	H30	C21	H32	105.61
C6	C7	H10	121.46	H31	C21	H32	105.05
C8	C7	H9	95.85	C20	C22	H33	104.51
C8	C7	H10	112.13	C20	C22	H34	113.03
H9	C7	H10	102.06	C23	C22	H33	102.79
C7	C8	H11	105.75	C23	C22	H34	111.60
C9	C8	H11	107.35	H33	C22	H34	107.84
C14	C8	H11	111.17	C22	C23	H35	110.70
C8	C9	H12	106.66	C22	C23	H36	117.77
C10	C9	H12	103.58	C24	C23	H35	104.90
C9	C11	H13	110.62	C24	C23	H36	106.00
C9	C11	H14	111.23	H35	C23	H36	104.77
C12	C11	H13	107.04	C23	C24	H37	105.39
C12	C11	H14	106.08	C23	C24	H38	106.85
H13	C11	H14	107.67	C25	C24	H37	114.24
C11	C12	H15	115.88	C25	C24	H38	109.86
C11	C12	H16	111.10	H37	C24	H38	104.77
C13	C12	H15	111.31	C24	C25	H39	112.61
C13	C12	H16	106.27	C26	C25	H39	102.26

H15	C12	H16	103.67	C27	C25	H39	112.16
C8	C14	H17	101.74	C25	C26	H40	113.66
C13	C14	H17	104.01	C25	C26	H41	114.21
C15	C14	H17	112.66	C25	C26	H42	113.99
C14	C15	H18	104.37	H40	C26	H41	104.55
C14	C15	H19	108.74	H40	C26	H42	105.14
C16	C15	H18	114.85	H41	C26	H42	104.24
C16	C15	H19	119.13	C25	C27	H43	100.03
H18	C15	H19	104.86	C25	C27	H44	112.49
C15	C16	H20	114.27	C25	C27	H45	108.38
C15	C16	H21	113.54	H43	C27	H44	109.66
C17	C16	H20	112.09	H43	C27	H45	106.63
C17	C16	H21	110.21	H44	C27	H45	118.00
H20	C16	H21	98.59				

Angles are in degree. Estimated standard deviations in the least significant figure are given parentheses.

Table - 3

Intramolecular distance involving the non-hydrogen atoms

Atom	Atom	Distance	Atom	Atom	Distance
CL1	C3	1.81 (2)	C11	C12	1.57 (2)
O1	C5	1.50 (2)	C12	C13	1.52 (2)
O1	C6	1.45 (2)	C13	C14	1.52 (2)
C1	C2	1.55 (2)	C13	C17	1.58 (2)
C1	C10	1.51 (2)	C13	C18	1.55 (2)
C2	C3	1.53 (2)	C14	C15	1.54 (2)
C3	C4	1.49 (2)	C15	C16	1.52 (2)
C4	C5	1.48 (2)	C16	C17	1.56 (2)
C5	C6	1.52 (2)	C17	C20	1.52 (2)
C5	C10	1.52 (2)	C20	C21	1.49 (2)
C6	C7	1.50 (2)	C20	C22	1.51 (2)
C7	C8	1.54 (2)	C22	C23	1.52 (2)
C8	C9	1.55 (2)	C23	C24	1.56 (2)
C8	C14	1.52 (2)	C24	C25	1.49 (2)
C9	C10	1.57 (2)	C25	C26	1.50 (3)
C9	C11	1.51 (2)	C25	C27	1.55 (2)
C10	C19	1.51 (2)			

Distances are in angstoms. Estimated standard deviations in the least significant figure are given parentheses.

Table - 4

Intramolecular bond angles involving the nonhydrogen atoms

Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle
C5	O1	C6	62 (1)	C5	C10	C19	105 (2)
C2	C1	C10	114 (2)	C9	C10	C19	113 (2)
C1	C2	C3	109 (2)	C9	C11	C12	114 (2)
CL1	C3	C2	107 (2)	C11	C12	C13	108 (2)
CL1	C3	C4	110 (3)	C12	C13	C14	108 (2)
C2	C3	C4	110 (1)	C12	C13	C17	113 (2)
C3	C4	C5	108 (2)	C12	C13	C18	112 (2)
O1	C5	C4	108 (2)	C14	C13	C17	100 (1)
O1	C5	C6	57 (1)	C14	C13	C18	113 (2)
O1	C5	C10	113 (2)	C17	C13	C18	110 (1)
C4	C5	C6	119 (2)	C8	C14	C13	114 (2)
C4	C5	C10	120 (2)	C8	C14	C15	120 (2)
C6	C5	C10	120 (2)	C13	C14	C15	104 (1)

O1	C6	C5	61 (1)	C14	C15	C16	104 (1)
O1	C6	C7	114 (2)	C15	C16	C17	108 (2)
C5	C6	C7	123 (2)	C13	C17	C16	102 (2)
C6	C7	C8	114 (1)	C13	C17	C20	122 (1)
C7	C8	C9	114 (1)	C16	C17	C20	111 (1)
C7	C8	C14	110 (2)	C17	C20	C21	111 (1)
C9	C8	C14	109 (2)	C17	C20	C22	111 (2)
C8	C9	C10	111 (2)	C21	C20	C22	110 (2)
C8	C9	C11	113 (1)	C20	C22	C23	116 (2)
C10	C9	C11	114 (2)	C22	C23	C24	112 (2)
C1	C10	C5	107 (1)	C23	C24	C25	115 (2)
C1	C10	C9	110 (2)	C24	C25	C26	110 (2)
C1	C10	C19	111 (2)	C24	C25	C27	112 (2)
C5	C10	C9	111 (2)	C26	C25	C27	107 (2)

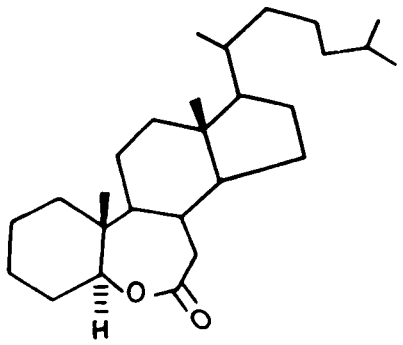
Angles are in degrees. Estimated standard deviations in the least significant figure are given in parentheses.

Due to the formation of oxirane ring in (XIV) bond angles around C5 and C6 undergo changes which are causing strain and geometrical deformation in rings A and B.

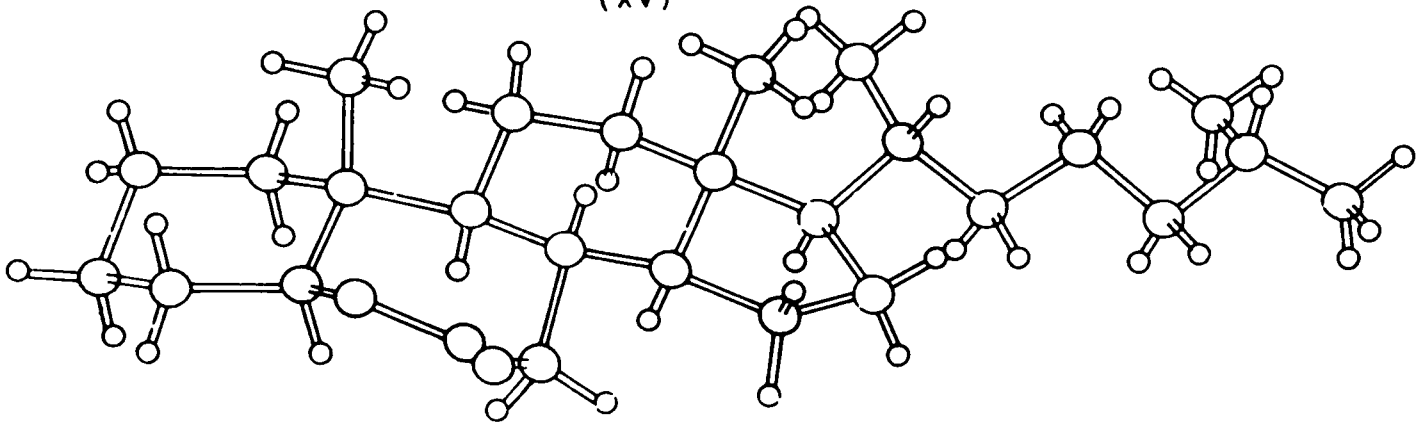
The X-ray crystal study of 6-oxa-B-homo-5α-cholestan-7-one (XV) :

During the preparation of oxasteroids, when 5α-cholestan-6-one (XXII) was treated with perbenzoic acid in chloroform (p-toluenesulphonic acid as catalyst), 6-oxa-B-homo-5α-cholestan-7-one[17] (XV) (Fig.11 a, b) was obtained which was characterized by (IR, ^{1}H-NMR, Mass). The assigned structure was further supported by X-ray crystallography. The results obtained were : molecular formula; $C_{27}H_{46}O_2$, molecular weight; 402.66, crystal morphology; colourless plate, crystal dimension (mm); 0.10 x 0.20 x 0.10, crystal system; monoclinic, Lattice parameters; a = 5.971 (2), b = 11.043(1), c = 19.243(1) A°, space group; $P2_1$, Z = 2 (two molecules per unit cell), Density (Dc); 1.062 g/cm^3, radiation; wkα (λ = 1.54178A°), temperature; 23 °C, structure solution; Patterson method.

The most important data obtained by X-ray crystallography are bond length and bond angle and different structural and conformational shape of the molecule.



(XV)



(Fig. 11a)

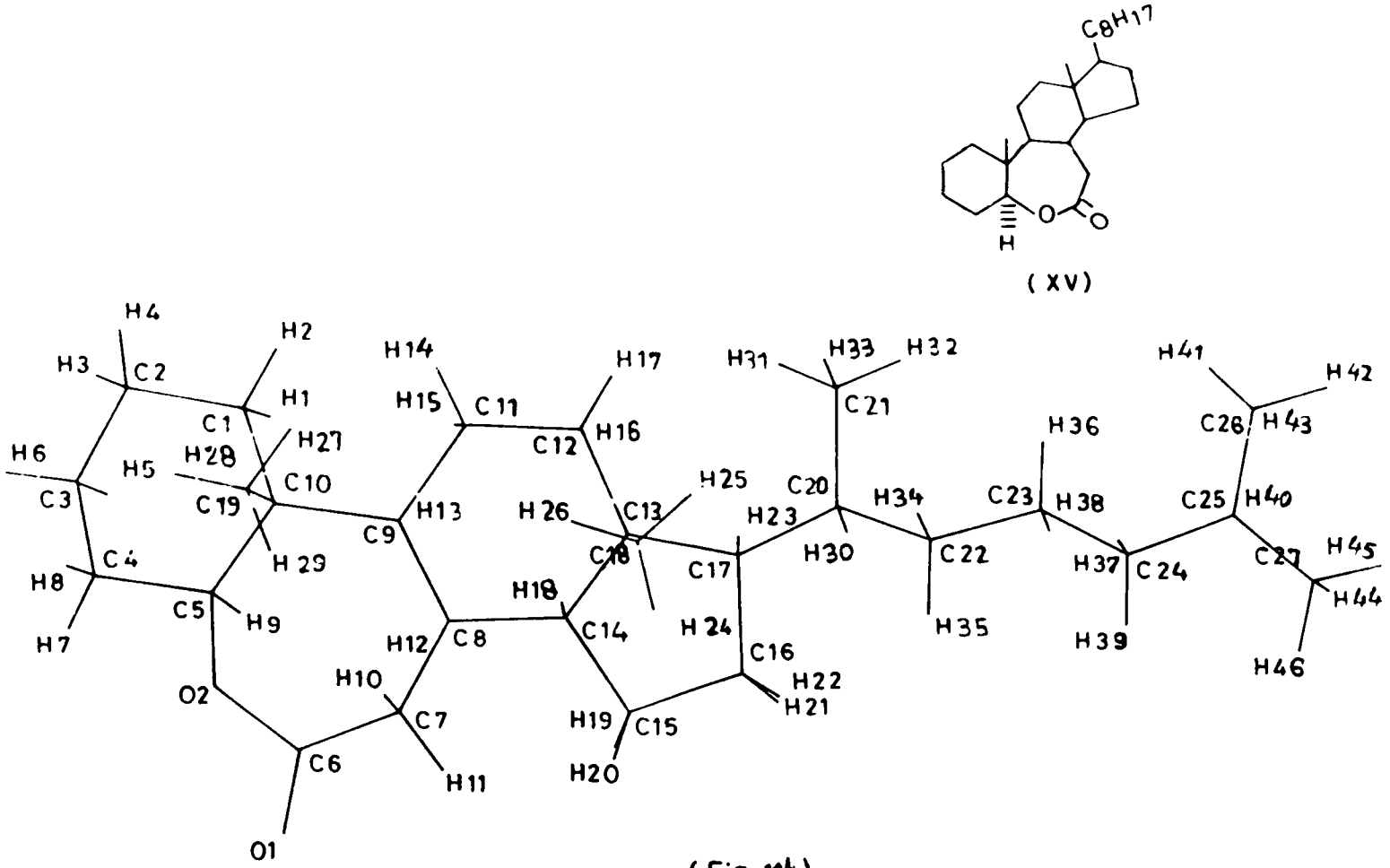
General view of the molecule

Table - 1

Intramolecular distances involving hydrogen atoms

Atom	Atom	Distance	Atom	Atom	Distance
O1	C6	1.21 (1)	C14	H18	0.970
O2	C5	1.47 (1)	C15	C16	1.54 (2)
O2	C6	1.36 (1)	C15	H19	0.911
C1	C2	1.51 (2)	C15	H20	0.999
C1	C10	1.56 (2)	C16	C17	1.56 (2)
C1	H1	0.980	C16	H21	0.970
C1	H2	0.913	C16	H22	0.969
C2	C3	1.54 (2)	C17	C20	1.53 (2)
C2	H3	0.910	C17	H23	0.964
C2	H4	0.954	C18	H24	0.950
C3	C4	1.53 (2)	C18	H25	0.950
C3	H5	0.953	C18	H26	0.921
C3	H6	0.943	C19	H27	0.966
C4	C5	1.51 (2)	C19	H28	0.912
C4	H7	0.936	C19	H29	0.924
C4	H8	0.905	C20	C21	1.52 (2)

C5	C10	1.53 (2)	C20	C22	1.54 (2)
C5	H9	0.973	C20	H30	0.964
C6	C7	1.51 (1)	C21	H31	0.964
C7	C8	1.55 (2)	C21	H32	0.947
C7	H10	0.978	C21	H33	0.956
C7	H11	0.954	C22	C23	1.50 (2)
C8	C9	1.53 (1)	C22	H34	0.928
C8	C14	1.53 (1)	C22	H35	0.997
C8	H12	0.980	C23	C24	1.52 (2)
C9	C10	1.59 (2)	C23	H36	0.938
C9	C11	1.56 (2)	C23	H37	0.917
C9	H13	0.973	C24	C25	1.51 (2)
C10	C19	1.55 (2)	C24	H38	0.873
C11	H14	0.943	C24	H39	0.995
C11	H15	0.940	C25	C26	1.48 (3)
C12	C11	1.51 (2)	C25	C27	1.39 (3)
C12	H16	0.935	C25	H40	0.925
C12	H17	0.944	C26	H41	0.878
C13	C12	1.54 (2)	C26	H42	1.009



(XV)

(Fig. 11b)

Plot showing atomic arrangement and numbering

C13	C17	1.54 (2)	C26	H40	0.954
C13	C18	1.56 (2)	C27	H44	0.939
C14	C13	1.50 (2)	C27	H45	0.993
C14	C15	1.53 (2)	C27	H46	1.015

Distances are in angstroms. Estimated standard deviations in the least significant figure are given in parentheses.

Table - 2

Intramolecular bond angles involving the hydrogen atoms

Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle
C5	O2	C6	122 (1)	C8	C14	C18	104.16
C2	C1	C10	116 (1)	C13	C14	H15	104 (1)
C2	C1	H1	108.44	C13	C14	H18	108.53
C2	C1	H2	109.50	C14	C15	C16	104 (1)
C10	C1	H1	105.95	C14	C15	H19	112.44
C10	C1	H2	107.25	C14	C15	H20	108.78
H1	C1	H2	109.97	C16	C15	H19	114.24
C1	C2	C3	110 (1)	C16	C15	H20	108.94

C1	C2	H3	110.45	H19	C15	H20	108.51
C1	C2	H4	108.42	C15	C16	H22	108.60
C3	C2	H3	109.14	C17	C16	H21	122.16
C3	C2	H4	106.09	C17	C16	H22	111.25
H3	C2	H4	112.70	H21	C16	H22	106.28
C2	C3	C4	108 (1)	C13	C17	C16	103 (1)
C2	C3	H5	111.53	C13	C17	C20	121 (1)
C2	C3	H6	110.34	C13	C17	H23	109.92
C4	C3	H5	109.71	C16	C17	C20	111 (1)
C4	C3	H6	106.94	C16	C17	H23	105.59
H5	C3	H6	109.83	C20	C17	H23	104.97
C3	C4	C5	111 (1)	C13	C18	H24	106.63
C3	C4	H7	107.19	C13	C18	H25	105.91
C3	C4	H8	108.40	C13	C18	H26	106.52
C5	C4	H7	108.40	H24	C18	H25	111.29
C5	C4	H8	106.87	H24	C18	H26	112.01
H7	C4	H8	114.82	H25	C18	H26	113.89
O2	C5	C4	103 (1)	C10	C19	H28	107.80
O2	C5	C10	113 (1)	C10	C19	H29	107.34

O2	C5	H9	102.54	H27	C19	H28	111.39
C4	C5	C10	116 (1)	H27	C19	H29	110.36
C4	C5	H19	108.57	H28	C19	H29	115.35
C4	C5	H9	112.93	C21	C20	H30	107.71
O1	C6	O2	114 (1)	C22	C20	H30	107.70
O1	C6	C7	127 (1)	C17	C20	C21	115 (1)
O2	C6	C7	119 (1)	C17	C20	C22	110 (1)
C6	C7	C8	110 (1)	C17	C20	H30	107.23
C6	C7	H10	109.38	C21	C20	C22	110 (1)
C6	C7	H11	104.28	C20	C21	H31	109.89
C8	C7	H10	111.37	C20	C21	H32	111.39
C8	C7	H11	114.80	C20	C21	H33	109.91
H10	C7	H11	106.85	H31	C21	H32	103.55
C7	C8	C9	113 (1)	H31	C21	H33	107.80
C7	C8	C14	106 (1)	H32	C21	H33	109.22
C7	C8	H12	108.19	C20	C22	C23	116 (1)
C9	C8	C14	108 (1)	C20	C22	H34	108.8
C9	C8	H12	110.70	C20	C22	H35	105.85
C14	C8	H12	109.95	C23	C22	H34	111.29

C8	C9	C10	119 (1)	C23	C22	H35	106.56
C8	C9	H14	111 (6)	H34	C22	H35	107.28
C8	C9	H13	103.18	C22	C23	C24	113 (2)
C10	C9	C11	110 (1)	C22	C23	H36	104.49
C10	C9	H13	105.07	C22	C23	H38	108.40
C11	C9	H13	107.81	C24	C23	H36	105.43
C1	C10	C5	107 (1)	C24	C23	H37	111.46
C1	C10	C9	107 (1)	H36	C23	H37	113.57
C1	C10	C19	110 (1)	C23	C24	C25	110 (2)
C5	C10	C9	110 (1)	C23	C24	H37	113.77
C5	C10	C19	111 (1)	C23	C24	H39	105.29
C9	C10	C19	111 (1)	C25	C24	H37	110.96
C9	C11	C12	115 (1)	C25	C24	H39	104.55
C9	C11	H14	106.15	H37	C24	H39	112.07
C9	C11	H15	105.70	C24	C25	C26	112 (2)
C12	C11	H14	109.10	C24	C25	C27	113 (2)
C12	C11	H15	110.05	C24	C25	H40	109.40
H14	C11	H15	110.96	C26	C25	H40	106.68
C13	C12	C11	110 (1)	C27	C25	H40	103.31

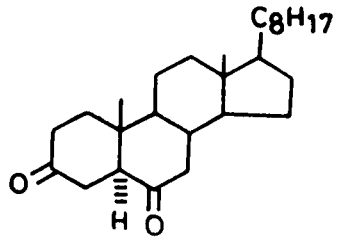
C13	C12	H16	111.50	C25	C26	H41	110.63
C13	C12	H17	107.50	C25	C26	H42	105.83
C11	C12	H16	109.10	C25	C26	H43	109.37
C11	C12	H17	107.04	H41	C26	H42	110.37
H16	C12	H17	111.27	H41	C26	H43	115.65
C14	C13	C12	109 (1)	H42	C26	H43	104.41
C14	C13	C17	102 (1)	C25	C27	H44	117.82
C14	C13	C18	114 (1)	C25	C27	H45	112.99
C12	C13	C17	116 (1)	C25	C27	H46	111.51
C12	C13	C18	110 (1)	H44	C27	H45	106.73
C17	C13	C18	107 (1)	H44	C27	H46	105.05
C8	C14	C13	115 (1)	H45	C27	H46	101.17
C8	C14	C15	118 (1)				

Angles are in degrees. Estimated standard deviations in the least significant figure are given in parentheses.

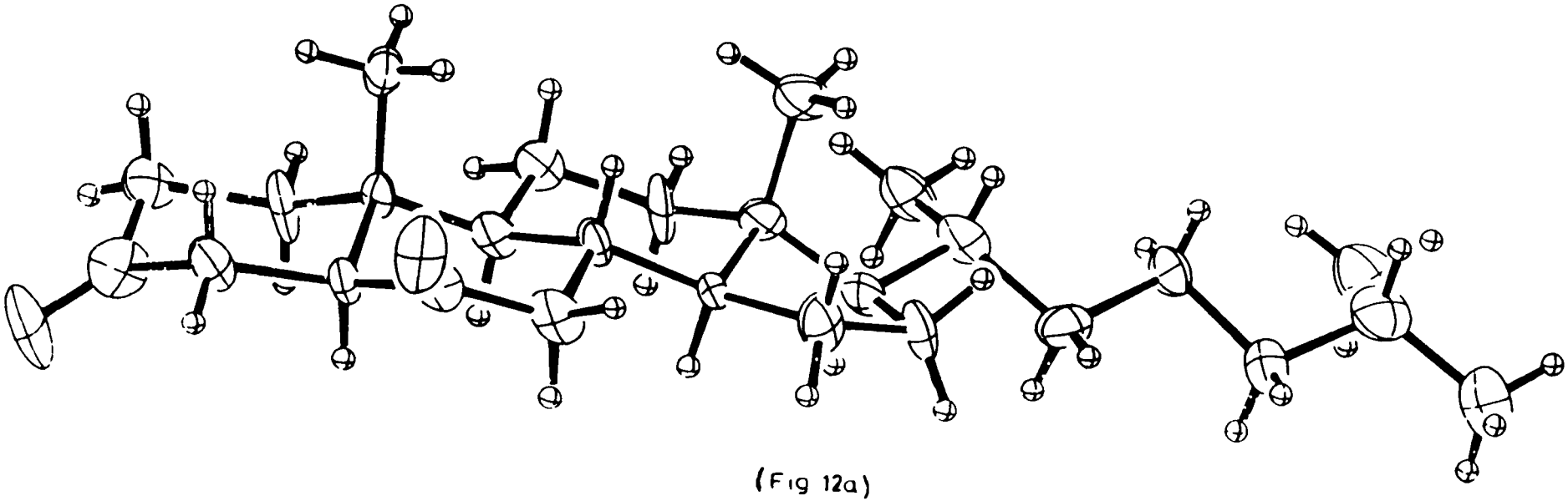
Due to ring enlargement from six-membered to seven membered and lactone moiety having sp^2 hybridized carbon lactone (XV) with tendency to have planar geometry suffers deformation in seven membered B-ring. It is pertinent to mention that ring C also suffers slight conformational change.

X-Ray crystal study of 5α-cholestane-3,6-dione(XVI) :

The most interesting compound involved in many synthesis of variety of steroidal compound is 5α-cholestane-3, 6-dione (XVI)[18] (Fig. 12a,b) This compound was prepared, characterized and its detail study of X-ray crystallography was done. The results are : molecular formula, $C_{27}H_{44}O_2$; molecular weight, 400; crystal morphology, colourless plate, crystal dimensions (mm); 0.20 x 0.50 x 0.30, crystal system; monoclinic, lattice parameters; a = 8.216 (3), b = 7.616 (2), C = 19.706 (3)A°, β = 92.86 (2)°, volume; 1231.6 (5) $A^{\circ 3}$, space group; $P2_1$ (≠ 4), Z= 2 (two molecules per unit cell), radiation; cμkα = 1.54178 A°, temperature; 23°C, structure solution; direct method. Bond lengths and bond angle of carbon – carbon and carbon hydrogen bonds can be known X-ray studies.



(XVI)



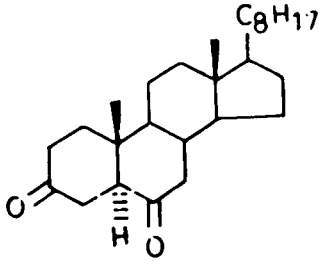
(Fig 12a)

General view of the molecule

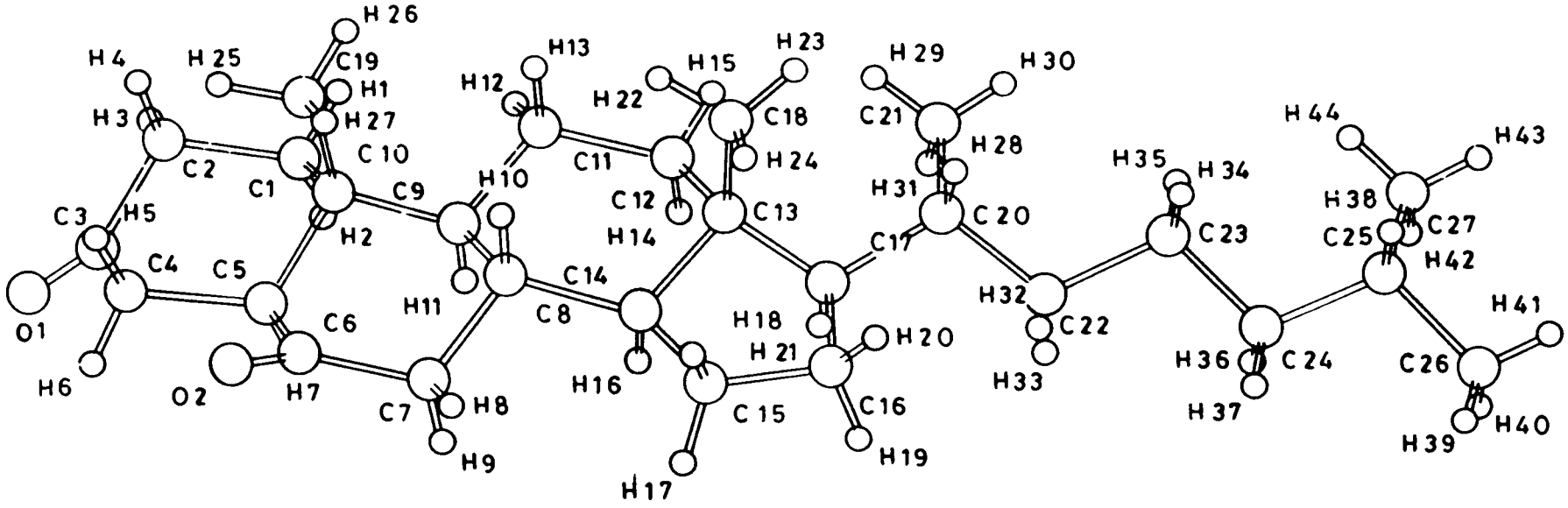
Table - 1

Intramolecular distances involving the hydrogen atoms.

Atom	Atom	Distance	Atom	Atom	Distance
C1	H1	0.952	C18	H23	0.957
C1	H2	0.948	C18	H24	0.948
C2	H3	0.951	C19	H25	0.950
C2	H4	0.949	C19	H26	0.952
C4	H5	0.950	C19	H27	0.947
C4	H6	0.949	C20	H28	0.949
C5	H7	0.949	C21	H29	0.950
C7	H8	0.949	C21	H30	0.951
C7	H9	0.949	C21	H31	0.950
C8	H10	0.951	C22	H32	0.950
C9	H11	0.950	C22	H33	0.949
C11	H12	0.951	C23	H34	0.949
C11	H13	0.950	C23	H35	0.952
C12	H14	0.950	C24	H36	0.950
C12	H15	0.952	C24	H37	0.948
C14	H16	0.949	C25	H38	0.950



(XVI)



(Fig. 12b)

Plot showing atomic arrangement and numbering

C15	H17	0.949	C26	H39	0.946
C15	H18	0.949	C26	H40	0.950
C16	H19	0.948	C26	H41	0.951
C16	H20	0.950	C27	H42	0.950
C17	H21	0.951	C27	H43	0.951
C18	H22	0.950	C27	H44	0.952

Distances are in angstroms. Estimated standard deviations in the least significant figure are given in parentheses.

Table - 2

Intramolecular bond angles involving the hydrogen atoms

Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle
C2	C1	H1	108.23	C16	C17	H21	107.60
C2	C1	H2	108.41	C20	C17	H21	10749
C10	C1	H1	108.44	C13	C18	H22	109.49
C10	C1	H2	108.55	C13	C18	H23	109.35
H1	C1	H2	109.48	C13	C18	H24	109.49
C1	C2	H3	109.15	H22	C18	H23	109.35

C1	C2	H4	109.30	H22	C18	H24	109.67
C3	C2	H3	109.28	H23	C18	H24	109.36
C3	C2	H4	109.40	C10	C19	H25	109.35
H3	C2	H4	109.40	C10	C19	H26	109.27
C3	C4	H5	108.79	C10	C19	H27	109.41
C3	C4	H6	108.80	H25	C19	H26	109.34
C5	C4	H5	108.91	H25	C19	H27	109.77
C5	C4	H6	108.92	H26	C19	H27	109.61
H5	C4	H6	109.57	C17	C20	H28	107.34
C4	C5	H7	106.53	C22	C20	H28	107.27
C6	C5	H7	106.64	C21	C20	H28	107.18
C10	C5	H7	106.48	C20	C21	H29	109.44
C6	C7	H8	108.50	C20	C21	H30	109.49
C6	C7	H9	108.48	C20	C21	H31	109.57
C8	C7	H8	108.45	H29	C21	H30	109.38
C8	C7	H9	108.39	H29	C21	H31	109.48
H8	C7	H9	109.60	H30	C21	H31	109.46
C7	C8	H10	107.82	C20	C22	H32	107.91
C9	C8	H10	107.84	C20	C22	H38	107.84

C14	C8	H10	107.87	C23	C22	H32	107.93
C8	C9	H11	106.01	C23	C22	H33	107.83
C10	C9	H11	105.92	H32	C22	H33	109.60
C11	C9	H11	105.87	C22	C23	H34	109.82
C9	C11	H12	107.63	C22	C23	H35	109.69
C9	C11	H13	107.69	C24	C23	H34	109.86
C12	C11	H12	17.57	C24	C23	H35	109.77
C12	C11	H13	107.63	H34	C23	H35	109.34
H12	C11	H13	109.39	C23	C24	H36	109.09
C11	C12	H14	108.54	C23	C24	H37	109.11
C11	C12	H15	108.45	C25	C24	H36	109.13
C13	C12	H14	108.54	C25	C24	H37	109.18
C13	C12	H15	108.52	H36	C24	H37	109.68
H14	C12	H15	109.30	C24	C25	H38	106.75
C8	C14	H16	104.54	C26	C25	H38	106.71
C13	C14	H16	104.52	C27	C25	H38	106.52
C15	C14	H16	105.75	C25	C26	H39	109.45
C14	C15	H17	111.28	C25	C26	H40	109.26
C14	C15	H18	111.38	C25	C26	H41	109.28

C16	C15	H17	111.29	H39	C26	H40	109.77
C16	C15	H18	111.40	H39	C26	H41	109.72
H17	C15	H18	109.67	H40	C26	H41	109.35
C15	C16	H19	110.11	C25	C27	H42	109.61
C15	C16	H20	109.98	C25	C27	H44	109.63
C17	C16	H19	109.97	C25	C27	H44	109.58
C17	C16	H20	109.84	H42	C27	H43	109.40
H19	C16	H20	109.63	H42	C27	H44	109.32
C13	C17	H21	107.48	H43	C27	H44	109.28

Angles are in degrees. Estimated standard deviations in the least significant figure are given in parentheses.

Table - 3

Intramolecular distance involving the non-hydrogen atoms

Atom	Atom	Distance	Atom	Atom	Distance
O1	C3	1.19(2)	C11	C12	1.57(2)
O2	C6	1.21(2)	C12	C13	1.53(2)
C1	C2	1.61(2)	C13	C14	1.56(2)

C1	C10	1.52(2)	C13	C17	1.44(2)
C2	C3	1.54(4)	C13	C18	1.58(2)
C3	C4	1.52(2)	C14	C15	1.53(2)
C4	C5	1.61(2)	C15	C16	1.51(2)
C5	C6	1.50(2)	C16	C17	1.52(2)
C5	C10	1.55(2)	C17	C20	1.57(2)
C6	C7	1.47(2)	C20	C22	1.56(2)
C8	C9	1.51(2)	C22	C23	1.54(2)
C8	C14	1.58(2)	C23	C24	1.51(2)
C9	C10	1.47(2)	C24	C25	1.56(2)
C9	C11	1.54(2)	C25	C26	1.55(2)
C10	C19	1.55(2)	C25	C27	1.46(3)

Distances are in angstroms. Estimated standard deviations in the least significant figure are given in parentheses.

Table - 4

Intramolecular bond angles involving the non-hydrogen atoms

Atom	Atom	Atom	Angles	Atom	Atom	Atom	Angles
C2	C1	C10	114(1)	C9	C11	C12	117(1)
C1	C2	C3	110(1)	C11	C12	C13	113(1)
O1	C3	C2	127(2)	C12	C13	C14	105(1)
O1	C3	C4	122(2)	C12	C13	C17	120(1)
C2	C3	C4	111(1)	C12	C13	C18	110(1)
C3	C4	C5	112(1)	C14	C13	C17	99(1)
C4	C5	C6	112(1)	C14	C13	C18	108(1)
C4	C5	C10	113(1)	C17	C13	C18	113(1)
C6	C5	C10	111(1)	C8	C14	C13	117(1)
O2	C6	C5	126(1)	C8	C14	C15	120(1)
O2	C6	C7	123(2)	C13	C14	C15	105(1)
C5	C6	C7	111(1)	C14	C15	C16	102(1)
C6	C7	C8	113(1)	C15	C16	C17	107(1)
C7	C8	C9	110(1)	C13	C17	C16	107(1)
C7	C8	C14	112(1)	C13	C17	C20	117(1)
C9	C8	C14	111(1)	C16	C17	C20	111(1)

C8	C9	C10	116(1)	C17	C20	C22	113(1)
C8	C9	C11	107.8(9)	C17	C20	C21	112(1)
C10	C9	C11	114(1)	C22	C20	C21	110(1)
C1	C10	C5	107(1)	C20	C22	C23	116(1)
C1	C10	C9	110(1)	C22	C23	C24	108(1)
C1	C10	C19	113(1)	C23	C24	C25	111(1)
C5	C10	C9	105(1)	C24	C25	C26	112(2)
C5	C10	C19	109(1)	C24	C25	C27	114(2)
C9	C10	C19	113(1)	C26	C25	C27	110(2)

Distances are in angstroms. Estimated standard deviations in the least significant figure are given in parentheses.

Due to sp^2 hybridization of carbonsC3 and C6, the respective portions (C2, C3, C4 and C5, C6, C7) acquiring planarity cause definite conformational deformity in both the rings A and B, their conformation became pseudo chair form.

EXPERIMENTAL

All melting points were observed on a Kofler hot block apparatus and are uncorrected. IR spectra were obtained in KBr unless otherwise specified and the IR values given are in cm^{-1}. ^{1}H-NMR spectra were run in $CDCL_3$ with Me_4Si as the internal reference and values are given in ppm (δ) (s, singlet: br, broad; d, doublet; dd, double doublet; t, triplet; mc, miltiplet centred at). Thin layer chromatographic (TLC) plates were coated with silica gel and sprayed with 20% aqueous solution of perchloric acid. Petroleum ether refers to a fraction of b.p. 40 – 60 °C. Silica gel (~20) was used for each gram of the material to be separated in column chromatography. All glass wares were heated in oven at a temperature range of 200-225°C for at least 8 hours prior to their use. The solvents and reagents were purified according to the literature procedure. Programs for crystal structure determination Shelxs 76 (university of Cambridge England, 1976) and Shelxs 86 (university of Gottingen, Germany, 1986) were used. All the calculations were carried out on a Magnum computer.

Reaction of cholesterol (XVIII) with pyridinium dichromate (PDC): Cholest-4-ene-3, 6-dione (XVII) :

To a solution of cholesterol (XVIII, 10 g) in N, N-di-methyl formamide (220 ml), pyridinium dichromate[19] (PDC, 44 g) was added and the reaction mixture was stirred at room temperature for 4 hours. The reaction was monitored with the help of TLC plates. After completion of the reaction, water (220 ml) was added and the reaction mixture was worked up with ether. The ethereal layer was washed with water, dilute hydrochloric acid, sodium bicarbonate solution (5%) and water and dried over anhydrous sodium sulfate and the solvents were evaporated.

Cholest-4-ene-3, 6-dione (XVII) :

Solvent of crystallization : methanol, Yield : (8.7 g), m.p. 124°C (reported[20], m.p. 122-123°C).

Analysis found	C, 81.50; H, 10.45
Required	C, 81.41; H, 10.55%
U.V.	λ max 250.8 nm
IR	ν max 1695 (C=O), 1605 cm^{-1} (-C=C-).

^{1}H-NMR : δ6.15 (s, 1H, C4-vinylic proton), 2.66 (dd, 1H, H-7β, Jae = 5 Hz, J gem = 13 Hz). 2.49 (mc, 2H, H-2α, β), 2.12 (m, 2H, H-1α, β), 2.02 (dd, 1H, H-7α, Jae = 5 Hz, J gem = 14 Hz), 1.15 (s, 3H, C10-CH_3), 0.71 (s, 3H, C13-CH_3), 0.96 and 0.84 (side chain methyl protons).

^{13}C-NMR : C1(39.1128), C2(39.4332), C3(199.121), C4(125.404), C5(161.652), C6(201.256), C7(46.7842), C8(34.1814), C9(50.9483), C10(39.780), C11(23.7689), C12(35.5058), C13(42.5102), C14(56.5220), C15(27.9819), C16(23.9421), C17(55.9282), C18(11.8727), C19(17.4875), C20(35.6480), C21(18.6250), C22(34.1814), C23(20.8545), C25(27.9819), C26(22.7939), C27(22.5342).

Mass : m/z 398($M^{+\bullet}$, 100), 397(18), 396(13), 384(12), 383(10), 382(14), 370(4), 369(3), 366(3), 284(5), 282(3), 270(3), 269(1), 266(3), 246(5), 245(3), 242(4), 240(2), 228(5), 226(4), 214(4), 212(4), 210(3), 204(3), 202(4), 200(5), 198(4), 190(5), 188(9), 184(5), 183(2), 182(4), 180(3), 176(5), 175(4), 174(14), 173(3), 172(10), 170(7), 164(8),

162(8), 160(12), 158(13), 153(18), 151(9), 140(7), 137(12), 136(64), 135(26), 134(24), 120(7), 118(24), 108(35), 106(41), 104(36), 100(18).

Reaction of cholest-4-ene-3, 6-dione (XVII) with N-bromo-succinimide in presence of benzoyl peroxide (catalyst) : 2α, 7α-Dibromocholest-4-ene-3, 6-dione (XIII) :

To a solution of cholest-4-ene3, 6-dione (XVII), (3g) in dry benzene (60ml), N-bromosuccinimide (2g) was added under refluxed condition in small portions over a period of 3 hours in presence of benzoyl peroxide (as catalyst). After complete addition, the reaction mixture was further heated for 1 hour and then the solvent was evaporated under reduced pressure. The residue so obtained was taken in ether. The ethereal layer was washed with water, sodium sulfite solution (5%) and water successively and dried over anhydrous sodium sulfate. Removal of the solvent provided a brown solid which was chromatographed over silica gel.

2α, 7α-Dibromocholest-4-ene-3, 6-dione (XIII) :

Elution : pet. ether : ether (8:1), solvent of crystallization : methanol, Yield : (1.52 g), m.p. 180°C[15].

Analysis found : C, 58.12; H, 7.15

Required : C, 58.27; H, 7.19%

U.V. : λ max 240.8 nm.

IR : ν max 1700 (C=O), 1610 (-C=C-) and 785 cm^{-1} (C-Br)

^{1}H-NMR : δ 6.32 (s, 1H, C4-H vinylic proton), 4.85 (dd, 1H, H-2β, Jae = 5 Hz, Jaa = 14 Hz), 4.4 (d, 1H, H-7β, Jae = 4.5 Hz), 2.8 (dd, 1H, H-1α, Jae = 5 Hz, Jgem = 14 Hz), 2.46 (t, H-1β, Jaa and Jgem = 14 Hz), 1.24 (s, 3H, C10-CH_3), 0.74 (s, C13-CH_3) 0.98 and 0.86 (side chain methyl protons).

^{13}C-NMR : C1(47.4651), C2(49.1342), C3(190.377), C4(126.544), C5(159.316), C6(193.994), C7(57.6270), C8(43.2928), C9(37.6693), C10(42.2525), C11(20.3693), C12(38.3228),

C13(43.4298), C14(51.8922), C15(23.7273), C16(27.750), C17(55.6450), C18(12.2957), C19(18.6197), C20(35.6335), C21(18.3919), C22(35.9798), C23(22.8054), C24(39.4325), C25(28.0040), C26(22.8054), C27(22.5396).

Mass : m/z 554 (M^{+}, 56) 554(56), 553(6), 552(7), 477(35), 475(33), 474(30), 473(14), 472(5), 460(4), 397(6), 396(12), 395(24), 394(8), 393(9), 380(4), 367(5), 362(9), 360(7), 352(4), 322(4), 320(6), 306(7), 288(10), 282(11), 280(4), 272(6), 268(11), 267(3), 255(4), 254(8), 253(4), 252(7), 250(6), 246(19), 244(7), 242(6), 240(9), 238(9), 228(9), 226(11), 225(8), 224(10), 223(5), 222(6), 220(4), 218(7), 214(17), 213(6), 212(16), 210(11), 209(7), 208(9), 206(7), 202(9), 201(8), 200(12), 199(6), 198(16), 197(7), 196(15), 195(7), 194(9), 191(3), 190(10), 189(8), 188(29), 187(9), 186(33), 185(9), 184(16), 183(8), 182(14), 181(9), 180(13), 179(7), 178(13),

177(14), 176(11), 175(25), 173(12), 172(35), 171(10), 170(25), 169(9), 168(18), 167(14), 166(24), 165(27), 164(32), 163(9), 162(21), 160(30), 159(16), 157(14), 156(22), 155(20), 154(37), 153(93), 152(30), 151(32), 150(20).

3β-Chlorocholest-5-ene (XIX) :

Freshly purified thionyl chloride (75 ml) was added gradually to cholesterol) (XVIII) (100 g) at room temperature. A vigorous reaction ensured with the evolution of gaseous products. When the reaction slackened, the reaction mixture was gently heated at a temperature of 50-60° on a water bath for 1 hour and then poured on to crushed ice-water with stirring. The yellow solid thus obtained was filtered under suction and washed several times with ice-cold water and air dried. Recrystallization of crude product from acetone gave compound (XIX) (95.5 g), m.p. 95-96° (reported[21], m.p. 96°). It gave positive Beilstein test and a yellow colour with tetranitromethane in chloroform.

3β-Chloro-5, 6α-epoxy-5α-cholestane (XIV) :

3β-Chlorocholest-5-ene (XIX) (11 g) in chloroform (100 ml) was treated with a solution of perbenzoic acid (1:1 mol equivalent) and left for 20 hours, at a temperature of -8°. The reaction mixture was then washed successively with ice-cold sodium bicrabonate solution (5%), sodium thiosulphate (5%) solution and again with water. Evaporation of the solvent yielded (XIV) as an oil which was crystallized from acetone as needles (8.1g), m.p. 89° (reported, m.p. 89.5-90.5°)[16] .

Cholest-5-ene (XX) :

3β-Chlorocholest-5-ene (XIX) (10 g, 24.72 m mol) was dissolved in warm amylalcohol (200 ml) and sodium metal (24 g) was added in small portions to the solution with continuous stirring over a period of 8 hrs. The reaction mixture was heated occasionally during the course of reaction in order to keep the sodium metal dissolved. The reaction was poured into water, acidified with hydrochloric acid and allowed to stand over night. A white crystalline solid thus obtained was filtered under suction and washed thoroughly with water, and air dried. Recrystallization of the crude material from acetone gave cholest-5-ene (XX) (7.2 g, 19.45 m mol), m.p. 94 - 95° (reported[22] m.p. 89 - 91°)

6-Nitrocholest-5-ene (XXI) :

A suspension of freshly powdered cholest-5-ene (XX) (6 g), in glacial acetic acid (50 ml) was stirred at room temperature for 10 minutes, fuming nitric acid (20 ml; d, 1.52) was added and sodium nitrite (12 g) was added in small portions in 1 hour with continuous stirring for 2 hours more. The temperature of the reaction mixture was maintained between 20-25°C by external cooling. The reaction mixture was then poured into ice cold water. A yellow solid separated was filtered under suction, washed thoroughly with water and air dried. Recrystallisation from methanol provided pure 6-nitro-cholest-5-ene (XXI), (3.5 g), m.p. 118°C (reported[23], m.p. 117-118°C).

5α-Cholestan-6-one (XXII) :

6-Nitrocholest-5-ene (XXI), (6.0 g) was powdered, dissolved in warm glacial acetic acid (120 ml) and zinc dust (12 g) was gradually added with shaking. The suspension was heated for 4 hours and water 12 ml was added during the course of reaction. The hot solution was filtered to remove zinc powder, cooled to room temperature and diluted with excess of water. The

precipitate thus obtained was taken in ether. The ethereal solution was washed with water, sodium bicarbonate solution (10%), again with water and dried over anhydrous sodium sulfate. Removal of the solvent provided an oil which on crystallization from methanol gave thin plates of ketone (XXII), (3.5 g) m.p. 96°C (reported[24] m.p. 98°C).

Baeyer Villiger oxidation of 5α-c holestan-6-one (XXII) : 6-Oxa-B-homo-5α-cholestan-7-one (XV) :

5α-Cholestan-6-one (XXII), (2.0 g) was treated with chloroform solution of perbenzoic acid (2.5 ml equivalent) and few crystals of p-toluene-sulphonic acid for 96 hrs. The progress of reaction was monitored by TLC. After the completion of reaction, the reaction mixture was poured into ice water and extracted with ether. The ethereal layer was washed with water, sodium bicarbonate solution (5%) and finally with water and dried over anhydrous sodium sulphate. Removal of the solvents gave a semi solid material (1.85 g) which was chromatographed over silica gel (~ 40 g).

6-Oxa-B-homo-5α-cholestan-7-one (XV) :

Elution : Pet. ether : ether (10 : 1). Solvent of crystallization : Petroleum ether, Yield : (0.85 g), m.p. 147-148° (reported[17,25] m.p. 148°).

Analysis found : C, 80.3o; H, 11.14

Required : C, 80.06; H, 11.44%

CD : $[\alpha]^{20}_{D} = +28°$

IR : ν max 1735 (C=O), 1270 cm^{-1} (C-O).

^{1}H-NMR : δ4.19 (dd, 1H, H-5α, Jaa = 15 Hz, Jae = 5.5 Hz), 2.50 (brs, 1H, C7aβ-H), 2.45 (d, 1H, C7aα-H, J=3.5 Hz)[25], 1.1 (C10-CH_3), 0.67 (C13-CH_3), 0.95 and 0.85 (side chain methyl protons).

^{13}C-NMR : C1(29.421), C2(25.317), C3(22.206), C4(39.441), C5-O-(83.860), -C6-O-(175.152), C7(39.783), C8(34.92), C9(55.622), C10(40124), C11(21.372), C12(38.228), C13(42.681), C14(58.732), C15(23.761), C16(27.979), C17(56.441), C18(11.799), C19(18550), C20(35.679), C21(12.276), C22(35.952), C23(24.535), C24(38.364), C25(27.979), C26(22.525) and C27(22.783).

Mass : m/z 402 ($M^{+\cdot}$,18.5), 387($M^{+\cdot}$ -CH_3, 7), 384 ($M^{+\cdot}$ -H_2O, 17.5), 374 ($M^{+\cdot}$ -CO, 12.5), 360 ($M^{+\cdot}$ -CH_2=C=O, 6), 359 (m/z 374 –CH_3, 13), 356 (m/z 384 –CO, 9), 318 (m/z 374 –C_4H_8, 100), 317 (m/z 318 –H; 21), 303 (16), 289 (5), 262 (7.5), 247 (8.5), 219 (6) and 178 (16).

3β-Acetoxycholest-5-ene (XXIII) :

A mixture of cholesterol (XVIII) (50 g, 51.8 m mol), pyridine (75 ml, freshly distilled over KOH) and freshly distilled acetic anhydride (50 ml) was heated on a steam bath for 2 hrs. The resulting brown solution was poured onto crushed ice-water mixture with stirring. A light brown solid was obtained, which was filtered under suction, washed with water until free from pyridine and air-dried. The crude product on recrystallization from acetone gave the pure acetate (XXIII)(45.0 g), m.p. 115-116° (reported[26], m.p. 116°).

3β-Acetoxy-6-nitrocholest-5-ene (XXIV) :

3β-Acetoxycholest-5-ene (XXIII) (5.0 g, 11.682 m mol) was covered with nitric acid (125 ml, sp. gr. 1.52). Sodium nitrite (5.0 g) was gradually added over a period of 1 hr. with continuous stirring. Slight cooling was also required during the course of the reaction, and the stirring was continued for additional 2 hrs. A yellow spongy mass separated on the surface of the mixture, it was diluted with cold water (100 ml) then a green coloured solution was obtained. The whole mass was extracted with ether. The ethereal layer was washed with water, sodium bicarbonate solution (5%) (until washing become pink) and water dried over sodium sulphate anhydrous. Removal of the solvent provided the nitrocompound (XXIV) as an oil which was crystallized from methanol with traces of acetone (3.5 g, 7.9 m mol), m.p. 104° (reported[27], m.p. 102-104°).

3β-Acetoxy-5α-cholestan-6-one (XXV) :

3β-Acetoxy-6-nitrocholest-5-ene (XXIV) (3.0 g, 6.772 m mol) was dissolved in glacial acetic acid (125 ml) by warming the mixture and zinc dust (6.0 g) was added in small portions with shaking. The suspension was heated under reflux for 4 hrs and water (6 ml) was added now and then during the course of the reaction. The reaction was monitored by TLC. After the

completion of the reaction the reaction mixture was extracted with ether. The ethereal layer was washed successively with water, sodium bicarbonate solution (10%) and water and dried over anhydrous sodium sulphate. Removal of the solvent provided an oil which was crystallized from methanol to obtain ketone (XXV) (1.5 g), m.p. 128-129° (reported[28], m.p. 127-128°).

3β-Acetoxy-5-bromo-5α-cholestan-6-one (XXVI) :

To a solution of 3β-acetoxy-5α-cholestan-6-one (2 g) in acetic acid (5 ml) and ether (18 ml), bromine solution (1.1 g, bromine in 22 ml acetic acid) was added with shaking during 1 hour at 0°. Few drops of hydrobromic acid was added to catalyse the reaction. The bromo compound (XXVI) thus precipitated out was filtered and recrystallized from chloroform – ether (1.2 g) m.p. 162-64° (reported[18], m.p. 163-64°).

3β-Acetoxycholest-4-en-6-one (XXVII) :

A solution of 3β-acetoxy-5-bromo-5α-cholestan-6-one (XXVI) (2 g) and pyridine (20 ml) was heated under reflux for 8 hrs. under anhydrous conditions. The reaction mixture was poured into ice cold water, acidified with dilute hydrochloric acid and extracted with ether. The ethereal solution was washed successively with water, sodium bicarbonate solution (10%) and

water and dried over anhydrous sodium sulphate. Removal of the solvent provided an oil which was crystallized from methanol to obtain ketone (XXVII) (1.5 g), m.p. 107-109° (reported[18], m.p. 110°).

5α-Cholestane-3, 6-dione (XVI) :

A mixture of 3β-acetoxycholest-4-en-6-one (XXVII) (1 g), potassium hydroxide in methanol (4%, 3 ml) was heated under reflux for 1 hr. Half of the alcohol was removed under reduced pressure, when the dione (XVI) started crystallizing out. The solid was filtered under suction, washed several times with water, air dried.

5α-Cholestane-3, 6-dione (XVI) :

Solvent of crystallization : ethanol, Yield : (0.75 g), m.p. 168° (reported[18] 169°).

Analysis found : C, 81.1; H, 11.2

$C_{27}H_{44}O_2$ requires : C, 81.0; H, 11.0%

IR : ν max 1710 cm^{-1} (C=O)

^{1}H-NMR : δ2.1-2.7 (mc, C2-H_2, C5 -αH, C7 –H_2), 1.2 (C10 CH_3), 0.70 (C13 –CH_3), 0.98 and 0.88 (side chain methyl protons).

Mass : m/z 400 ($M^{+\bullet}$; 100), 385 ($M^{+\bullet}$ -CH_3; 40), 382 ($M^{+\bullet}$ -H_2O; 4.6).

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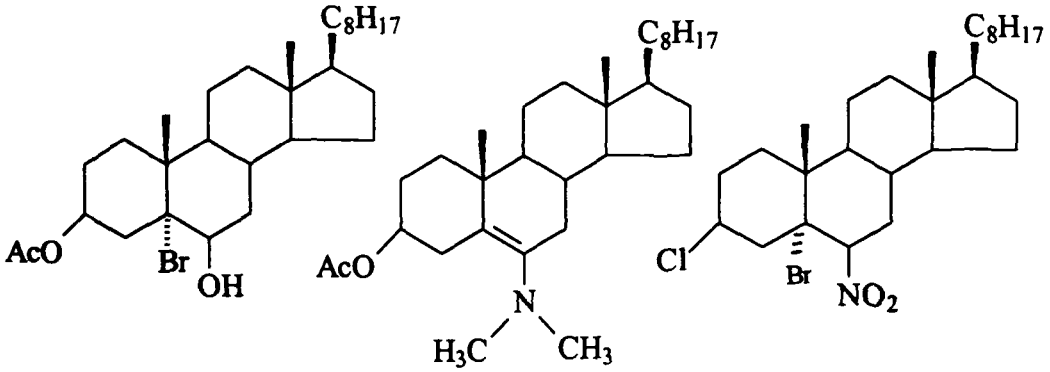
CHAPTER - 5

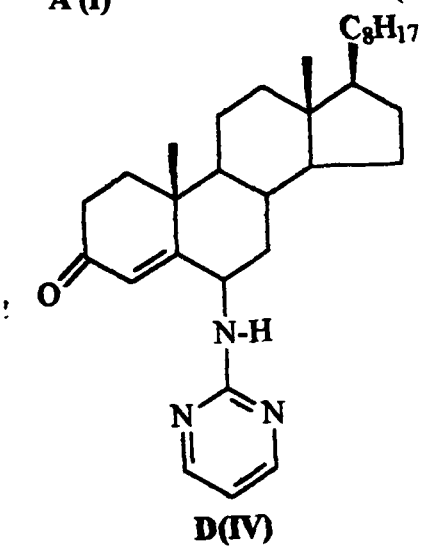
Neurotoxicological Effects of Steroidal compounds on lipid metabolism in different regions of rat brain.

THEORETICAL

Steroidal drugs, hormones and other biologically active steroids possess pronounced and specific biological activities. These steroids play important role in metabolism and therapeutics. The knowledge of properties of these steroids stimulated us to intensify research on their synthetic modification. Cholesterol molecule is the basic skeleton of all steroidal drugs and hormones of natural and synthetic origin. Synthetic drugs have been prepared by doing modification in the structure of natural steroidal molecule or alteration in the basic cholesterol nucleus, it involves the introduction of hetero atom at various sites in steroidal nucleus[1,2]. This alteration is always aimed to enhance the selectivity of these new derivatives in certain parameters of their biological activity.

To fulfill this aspiration an attempt has been made to test some less complicated easily synthesized cholesterol derivatives reported in the past, while exposing these derivatives on albino rats brain exclusive and elaborative biochemical, pharmacological, behavioural and histopathological studies have to be conducted steroids used in this study are : 3β-acetoxy-5 bromo-6β-hydroxy-5α-cholestane A (I) 3β-Acetoxy-6-dimethylamino cholest-5-ene B (II) 3β-Chloro-5-bromo-6β-nitro-5α-cholestane C (III) and 6β-Aminopyrimidinocholest-4-en-3-one D (IV)[2a,b,c].





Literature lacks such type of previous studies or trial of these derivatives for the search of new steroidal drugs. Present research work is aimed to achieve this motive, work done in this study is based on the facts described below.

Steroids are known to possess maximum receptors in the central nervous system, CNS of all vertebrates is highly enriched in various lipid[3]. They play vital role in both the structure and function of neural membrane. These lipids are metabolically very active and thus their functional

significance should not be overlooked. Studies on lipid profile in the CNS forms an important part of neurobiochemical investigations.

Present research work is confined only upto critical biochemical evaluation of total lipid and lipid fraction in major brain parts of albino rat (cerebrum, cerebellum, brain stem). Our second motive is to obtain detailed critical data based on structure activity co-relationship of cholesterol derivatives taken for trial. A large number of heterosteroids have been synthesized and screened for their chemical, biological, therapeutic and industrial potentials. Modification is done by substituting uncommon substituent (hetero atoms) at different position at unusual carbon frame work of steroids, it resulted either in decrease or increase in certain biological and physiological properties[1,2,4]. Adrenal glands produce steroidal hormones which are physiologically significant to our body. Destruction diseases of adrenal gland result in their deficiency of adrenal steroids. Symptoms in such cases could be studied as a symbol of deficiency of adrenal steroids. Addison (1855) was the first scientist who first time studied such clinical syndromes resulting from the destruction diseases of adrenal glands. These experiments inspired to Brown Sequrad (1856) to do the pioneering experiments on the effect of adrenolactomy and concluded that "adrenal glands are essential to life".

By the third decade of this century it was generally recognized that the cortex rather than the medulla is the life maintaining portion of the gland. The complex nature of adrenocortical deficiency was dramatized in 1930s by partisan research group, oriented to study either the imbalance of electrolytes or the defects in carbohydrate metabolism present in the deficient state. Renal loss of Na^+ was convincingly demonstrated to be a characteristic of adrenocortical insufficiency by Harrop and associates[5] as well as by Loeb and co-workers[6]. Equally convincing was the demonstration of a depletion of carbohydrate stores[7]. Further more, hypoglycemia could be corrected by adrenocortical extracts[8]. Glucose and glycogen formed under influence of the adrenal cortex during fasting appeared to be derived from tissue protein. From these studies emerged the concepts of two types of adrenocortical hormones. The mineral corticoids which primarily regulate electrolyte homeostasis and the glucocorticoids which are concerned with carbohydrate metabolism. This concept of dichotomy of salt and sugar hormones (mineralocorticoids and glucocorticoids) has proved useful at the present time in modified form. In 1932, the neurosurgeon Cushing described the syndrome of hypercorticism bear his name (Cushing 1932). The cases Cushing described were those of pituitary basopohilism recognized subsequently to be a consequence of hypersecretion of ACTH.

The preparation of adrenocortical extracts with a reasonable degree of activity was first accomplished in 1930. By 1942, organic chemists had isolated, crystallized and elucidated the structure of 28 steroids from the adrenal cortex[9]. Five of these compounds Cortisol (Hydrocortisone, Cortisone, Corticosterone, 11-Dehydrocorticosterone and 11-Desoxycosterone) were demonstrated to be biologically active. Another decade passed before the principal mineralocorticoids was discovered in 1950. Deming and Leutscher made an attempt to find the principal active material and discovered that the extracts of urine from patients with edema induced Na^+ retention and K^+ excretion in adrenolactomized rats. The definite evidence for the source of the active material was provided[10] who purified the compound with this activity from adrenocortical extracts. The substance was crystallized. The structure was established and the hormone was named aldosterone[11].

In this same era the role of adenohypophysis was being elucidated by other investigators. The classical studies of Foster and Smith 1926 established the fact that hypophysectomy results in atrophy of adrenal cortex. By 1933, it had been demonstrated that cell free extracts of the anterior pituitary has a stimulating effect upon the adrenal cortex of the hypopysectomized animal. Further chemical fractionation of such extracts led to the isolation of a hormone, ACTH, that acted selectively to cause chemical and morphological

changes in adrenal cortex. Its structure was established[12]. The rate of release of ACTH from adeno hypopysis was shown to be determined by the balance of the adrenal cortex and the excitatory effects of the nervous system.

A detailed analysis of the morphology of the adrenal cortex[13,14] suggested that the specific function of Zona glumerulosa is to autonomously elaborating a hormone regulated electrolyte balance. This hormone is now known to be aldosterone. Prolonged administration of sexual adrenocortical and thyroid hormones produces a feed back reaction causing diminished secretion of the corresponding glandotrophic hormones and also bring about morphological changes. This changes could be seen histologically discernable in the glandular parenchyma of the adenohypopysis.

In 1949, Hench demonstrated the effects of cortisone and ACTH in the treatment of rheumatoid arthritis. As early as 1929 Hench had been impressed by the fact that arthritis patient when pregnant or Jaundiced, experienced a temporary remission, he believed that a metabolite was responsible for the remission. The possibility that the antirheumatic substance might be an adrenocortical hormone was entertained and as soon as cortisone was available it was tested in a case acute rheumatoid arthritis. Fortunately an adequate dose was employed and the response was dramatic. Thereafter the detailed salutary effects of ACTH were also demonstrated[15]. The Noble prize

in medicine was Jointly awarded to Kendall and Reichstein (who were responsible for much of the basic chemical research that led to the synthesis of the steroids) and to Hench, whose contribution has just been described.

According to the British Medical Association reports (1968 on therapeutic abortion cited in the famous modies book of medical jurisprudence and toxicology), "Long term exposure of oral progestrins, androgens and estrogen causes foetal abnormality". The medical termination of pregnancy act, 1971 also legalize the abortion of the pregnant women, treated with steroidal drugs like cortisone or oral contraceptives[16].

Contrary to above report recent study reveals that antenatal corticosteroids given for a short period (24 hr and 1 week before birth) reduces respiratory distress syndrome, neonatal mortality by about 50% with comparable reduction in necrotising enterocolitis.

The benefits of antenatal corticosteroids are likely to out weigh any Possible disadvantage due to maternal or neonatal infections. There is no evidence of major adverse neurodevelopmental out come in later childhood among infants of treated mother[17a,b]. Due to this protective effect academic and professional bodies have recommended that with very few contraindications, the use of antenatal corticosteroids may be considered in women likely to deliver prematurely (Royal College meet 1993, NIH 1995).

Since these are the most effective single strategy for reducing the adverse consequences of preterm birth[18].

Steroidal therapy has been used extensively in head injury its effectiveness has documented in the work of Reulen et al. (1972)[19]. Various high potency glucocorticoids chiefly dexamethasone have been used widely in the management of intracranial hypertension and brain edema. Use of these steroids may dramatically and rapidly reduces the focal and general signs of brain tumour[20]. It has been confirmed by recent studies that combined oral contraceptive improve in the both acne and hirsutum. Role of parathyroid hormones and dihydrolachysterol is established in the reduction of lead toxicity from the bone. These hormones mobilize lead from the skeleton and augment the concentration of lead in blood and the rats of its excretion in urine[21]. In case of delayed neuropathy caused by organophosphorus compounds treatment with corticoid in such cases modify it for example delayed neuropathy caused by triortho – tolyl phosphate (TOTP), but the same treatment could not showed protective effect in the case of O-O-Di-isopropyl phosphorofluoridate (DFP) induced delayed neurotoxicity .It is because due to intrinsic differences between the organo phosphorus compound DFP and TOTP.

Sex linked factors have been found to affect rheumatoid arthritis (RA) and multiple sclerosis (MS) in a similar way but both diseases are slightly more common among women than man. During Pregnancy, in particular during the last trimester condition of both RA and MS tend to become better but again become painful in post partum[22]. Reason behind these changes are complex. Sex hormones or sex determined neuroendocrine factors may be involved here[23]. Collaegen induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE) are different models and affect different target organs but they are both dependent on autoaggressive T-Cells. Studies have proved that estrogen suppressess the pregnancy induced EAE and the Collagen induced arthritis in mice[24].

A lot of artificially synthesized hormones are being used in the general treatment, such as NS-3, an analogue of thyrotropin releasing hormone. Attempts are being done to produce safer and more effective steroids by modifying their structure or incorporating them with other drugs[25].

Synthetic glucocorticoids are widely used therapeutically for their immunosuppressive and antiinflammatory effects[26]. Use of the adrenal steroids exert opposite effects on immune system in the mammalians[27]. Allauddin and M. Smith (1962)[28], M. Smith and M. F. Surgue (1964)[29] have critically reviewed the biological activities in steroids possessing N-atom both

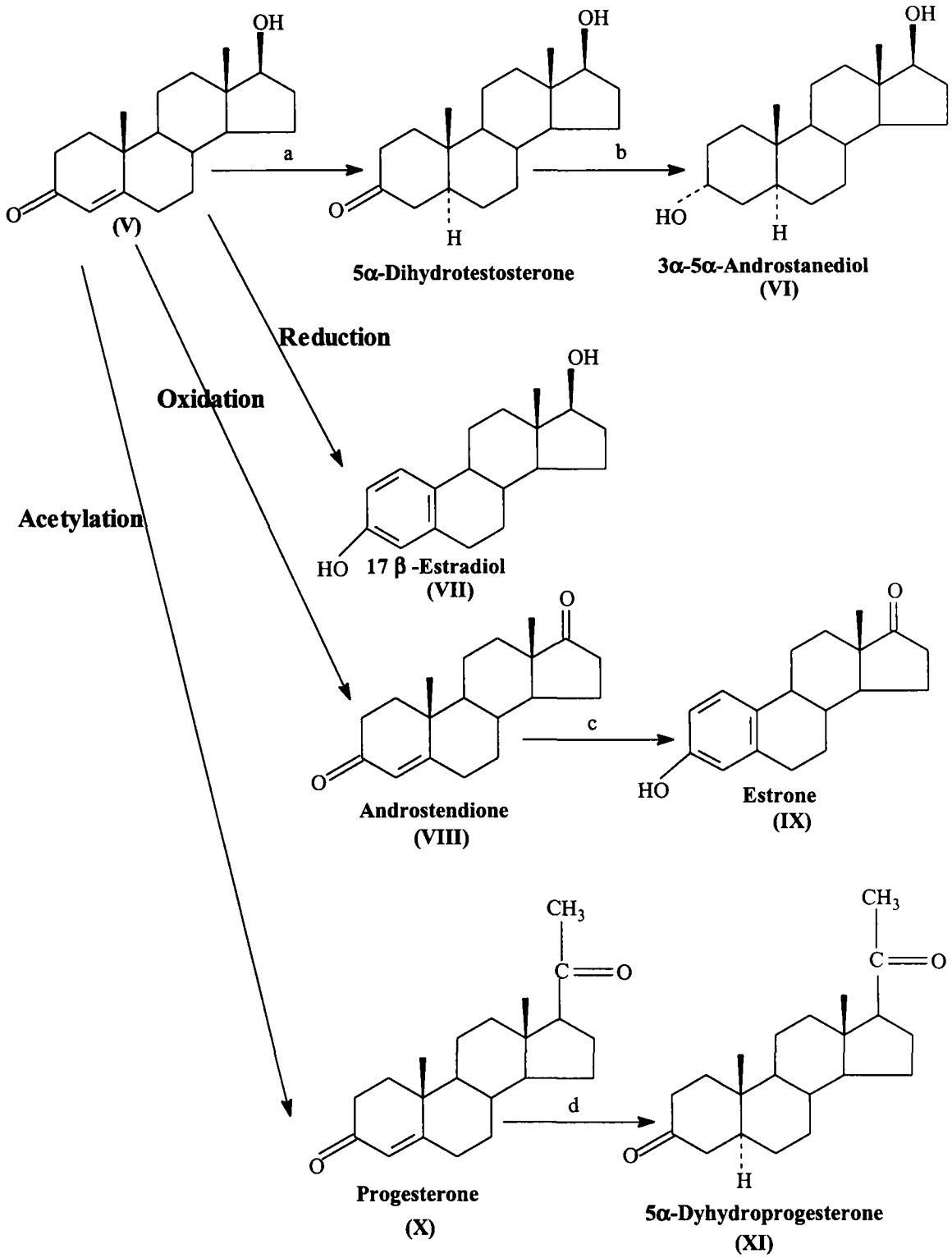
of natural and synthetic origin. At present steroidal drugs are the only drugs which fall under the category of life saving drugs. These drugs are used in highest medical emergency to save the life of million of sufferer everyday, steroidal drugs are commonly used on the occassions where generally any other non-steroidal drug fail to produce any curable effect. A lot of research work have been done in the field of hetero steroidal synthesis. Their identification has been done by chemical and spectral studies[30,31,32].

Steroidal drugs have been used for a wide range of diseases, for example, they are used as antiviral[33], antiallergic[34], anticonvulsant[30], antiulcer[35], analgesic[36], antifungal[33], antihypertensive[30], antifertility[37], nerve exciting[38], antimineralo[39], antibacterial[40], fungicidal[40], antithyroid[41,42,43] and growth inhibitor[44], in cardiac diseases[45], in skin disorders and as inflammation inhibitors[46]. The most alarming international problem of population out burst is being tackled by steroidal drugs. Even though a lot of alternate nonsteroidal drugs devices and methods have invented to avoid the conception, still steroidal drugs are the first and favourite choice among the family planners.

When the steroidal hormones interact with the target tissues, these gets metabolically transformed into more or less active metabolites. For the hormones like androgen and testosterone. These transformations appear to be of particular importance, this also suggests that these steroids may be a

perhormones. The brain, like the seminal vesicles, is able to convert testosterone to 5α-dihydrotestosterone (DHT) and 3α-5α-androstanediol V and VI) and also like the placenta converts testosterone to estradiol (VII) in scheme – 1.

ANDROGEN HORMONES :



Scheme - 1

Conversion never occurs equally in all brain regions. Regional distribution of 5α-reductase activity towards testosterone in rat brain is found in mid brain and brain stem with intermediate activity in hypothalamus and thalamus and lowest activity in cerebral cortex. The pituitary has higher 5α-reductase activity than any region of the brain and its activity is subjected to change as a result of gonadectomy, hormone replacement and post natal age. 5α-Dihydrotestosterone has been implicated in hypothalamus and pituitary as a potent regulator to of gonadotropin secretion but is relatively inactive towards male rat sexual behaviour[47].

It is interesting that progesterone (X) inhibits 5α-reductase activity towards (3H) testosterone and that (3H) progesterone is itself converted to (3H) 5α-dihydroprogesterone (XI). Progesterone competition for the 5α-reductase may explain some of the antiandrogenicity of this steroids[48]. The aromatization of testosterone to form estradiol (VII) and of androstenediols (VIII) to form estrone (IX) has been described in brain tissue in vitro and in vivo[49,50]. Aromatization is higher in hypothalamus and limbs structure than in cerebral cortex or pituitary gland in noncastrated animals is higher in male than in female brains. Aromatization has been found in brains of reptiles and amphibia as well as in mammals[51,52]. The capacity to aromatize testosterone and related androgens may therefore be a general property of vertebrate

brains. The functional role of aromatization has been studied most extensively in the rat, male sexual behaviour is facilitated by estradiol and testosterone[53]. Facilitation of male sexual behaviour can be blocked by these steroids[54]. Similar situation exists in birds, amphibia and reptiles, testosterone and estradiol can stimulate hetero typical sexual behaviour in male and females. Curiously, not all mammals are like the rat. For example, male sexual behaviour of the guinea pig and rhesus monkey is restored by nonaromatizble androgens androstenendione and dihydrotestosterone[55]. A number of other steroids transformation occur in brain tissue, but this metabolism does not appear to be of importance for interaction of those hormones with putative receptor sites. It has been found that both (3H) estradiol and (3H)-corticosterone are recovered from their binding sites in brain[56].

Corticosteroids have numerous wide spread effect on living system, they influence carbohydrate, protein and lipid metabolism, electrolyte and water balance and the functions of cardiovascular system, kidney, skeletal muscles, nervous system and other organs and tissues. Further more the corticosteroids endow the organism with the capacity to resist many types of nexious stimuli and environmental changes. In the absence of the adrenal cortex, survival is possible but only under the most rigidly prescribed condition for example, food must be available regularly, sodium chloride

ingested in the relatively large quantities and environmental temperature maintained with in a suitably narrow range. A given dose of corticosteroids may be physiological or pharmacological depending on the environment and activities of the organism. Under favourable condition, a small dose of corticosteroids maintains the adrenalectomized animal in a state of well being. Under adverse condition a relatively large dose is needed, if the animal is to survive. This same large dose given repetitively under optimal condition induces hypercorticism that is sign of corticosteroids excess. The function in the secretary activity of a normal subject are presumed to reflect the body's varying requirement for corticosteroids. The action of corticosteroids are often complexly related to the function of other hormones. For example in the absence of lipolitic hormones, cortisol, even in large concentration has virtually no effect on the rate of lipolysis in adipose tissue in vitro. Likewise, a sympathomimetic amine become evident. The necessary but not sufficient role of corticosteroids acting in concert with other regulatory forces has been termed "Permissive".

Estimates of the potencies of naturally occurring and synthetic corticosteroids in the categories of Na^+ retention (Reduction of Na^+ excretion by the kidney of the adrenalectomized animal), hepatic deposition of glycogen in fasted andrenalectomized animals and inflammatory effect (inhibition of the action of an agent that induces inflammation) are presented in Table-1. It

should be noted that such values are not fixed ratios but very considerably with the condition of the bioassays used. Potencies of steroids as judged by their ability to sustain life in the andrenalectomized animal closely parallel those determined for Na^+ retention. Potencies based on deposition of linear glycogen antiinflammatory effect, work capacity of skeletal muscles and involution of lymphoid tissues closely parallel one another. The corticosteroids have been classified in to mineralocorticoids and glycocorticoids, according to the potencies in the two categories[57].

TABLE - 1

Relative Potencies of Corticosteroids

	Na^+ Retention	Hepatic Glycogen Deposition	Anti-inflammatory effect
Natural Steroids			
Cortisol	1*	1	1
Cortisone	0.8*	0.8	0.8
Corticosterone	15	0.35	0.3
11-Desoxy Corticosterone	100	0.00	0.00
Aldosterone	3000	0.3	?
Synthetic Steroids			
Prednisolone	1*	4	4
Triamcinolone	0.00	5	5

* Promotes excretion of Na^+ under certain circumstances. Glucocorticoids are widely used therapeutically for their immunosuppressive and anti-inflammatory effects.

Adrenal steroids have been found to facilitate a form behavioural adaptation. In the absence of external stressors adrenal steroids are also secreted in varying amounts accounting to the time of day. In nocturnally active animals as the rat and in animals such as human being which are active during the day, the peak of this basal secretion always occurs near the end of the sleep period. Thus it is conceivable that adrenal steroid secretion may modulate behaviour as a function of the time of day. Indeed it has been reported that adrenal steroids modify the detection and recognition thresholds for a variety of sensory stimuli and influence the occurrence of the so-called rapid eye movement or paradoxical phase of sleep[58,59]. Desoxy corticosterone, the prototype of the mineralocorticoids is highly potent in regard to Na^+ retention but without effect on hepatic glycogen deposition cortisol the prototype of the glucocorticoids is highly potent in regard to linear glycogen deposition but weak in regard to Na^+ retention. The naturally occurring corticosteroids, cortisol and cortisone, as well as synthetic corticosteroids such as prednisolone and triamcinolone, are classified as glucocorticoids. However corticosterone is a steroids that has modest but significant activities in both

categories. In contrast, aldosterone is exceedingly potent with respect to Na^+ retention but has only modest potency for linear glycogen desposition. At rates secreted by the adrenal cortex or in doses that exert maximal effect on electrolyte balance, aldosterone has no significant effect on carbohydrate metabolism, it is thus classified a mineralocorticoids[57].

Change in the molecular structure may bring about changes in biological potency as a result of alteration in absorption protein binding rate of metabolic transformation, rate of excretion ability to traverse membrane and intrinsic effectiveness of the molecule at its site of action, in the follow paragraph, modification of the pregnane nucleus that have been of value in therapeutic agent are described in following figures. Following list show the effect of the modification discussed relative to cortisol.

Table - 2

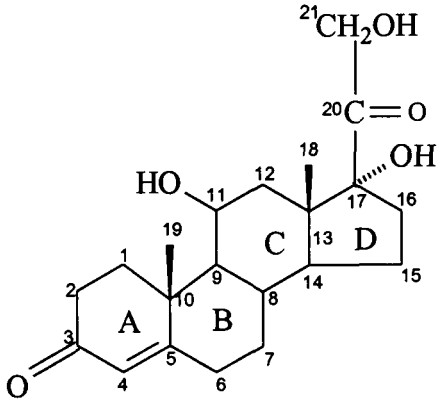
Relative Potencies And Equivalent Doses Of Corticosteroids.

Compound	Relative Antiinflammatory Potencies	Relative Retaining Na^+ Potency	Duration of action	Approx. Equiv. Dose (mg)
Cortisol (Hydrocortisone)	1	1	S	20
Tetra Hydrocortisol Prednisone	0	0	-	-
(^'-Cortisone) Prednisolone	4	0.8	1	5
(^'-Cortisol)	4	0.8	1	5
^6α-Methyl Prednisolone	5	0.5	1	4
Flurocortisone (9α-Flurocortisol)	10	125	S	-
11-Desoxycortisol	0	0	-	-
Cortisone (11-Dehydrocortisol)	0.8	0.8	S	25
Corticosterone	0.35	15	S	-
Triamcinolone (9α-fluro-16α-hydroxy Prednisolone)	5	0	1	4
Betamethasone (9α-fluro-16β-methyl Prednisolone)	25	0	L	2
Dexamethasone (9α-fluro-methyl Prednisolone)	25	0	L	0.75
Paramethasone (6α-fluro-16α-methyl Prednisolone)	10	0	L	0.75

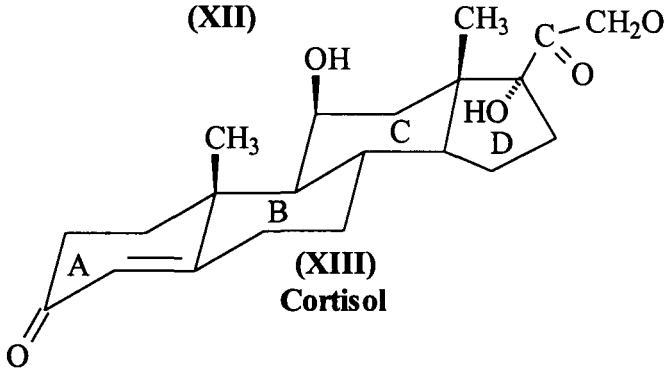
*S = Short or 8 to 12 hour biological half life.

I = Intermediate or 12 to 36 hour biological half life.

L = Long or 36 to 72 hour biological half life.



(XII)



(XIII)
Cortisol

These dose relationship apply only to oral or intravenous administration; relative potencies may differ greatly when injected intramuscularly or into joint spaces.

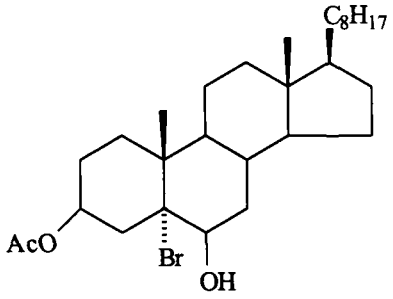
DISCUSSION

Two groups (controlled and experimental) each of 6 male albino rats of the average weight 200 ± 20 grams were separately taken for each steroidal derivative. Intraperitonial injection (3.0 mg/kg body weight of steroidal derivative dissolved in peanut oil) were given daily for 10 days, on 11th day animals were sacrificed for biochemical studies. The concentration of total lipids, cholesterol, gangliosides and rate of lipid peroxidation was determined in major brain parts cerebrum, cerebellum and brain stem.

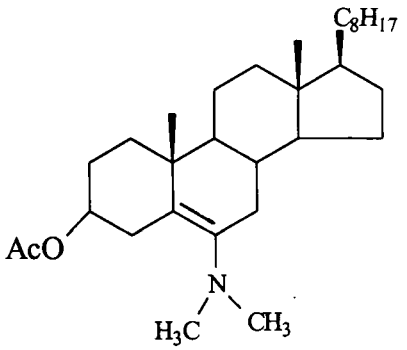
TOTAL LIPIDS :

The major structural and functional constituent of biological membrane are lipids, they also serve as essential component of several crucial enzyme systems, as fuel molecules as highly concentrated energy store[60]. In mammalian central nervous system, lipids comprise over half of the dry weight[3]. Almost all the lipids in CNS are found in membranes of cells. Different types of membranes accumulate different types of lipids. These brain lipids are constantly being synthesized replacing other molecules in membranes[61]. Lipids in the brain modulate the structure fluiding and of function of the biomembrane[62]. The brain lipids constitute components of ion channels and neurotransmitter receptors. On the other hand, they are also

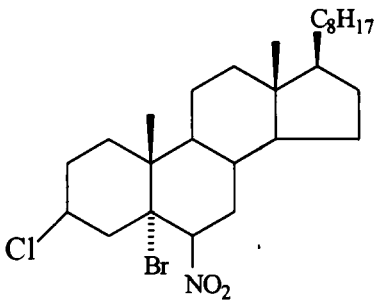
major constituents of myelin[63]. Lipids in the brain makes upto 65% of the dry weight of the white matter and 34 – 40% of gray matter[64]. Distinct regional differences occur in lipids contents, turn over in cell types and various pathways and centers of the brain[65]. Changes in the level of total lipids in the brain may occur by various physiological and chemical stressors and aging[66,67]. Changes in lipid fractions are induced by the steroids estrogen and progesterone in discrete brain parts[68]. In the present study an attempt has been made to study the elevation, deprivation in the level of total lipids in the various parts of brain in albino rats. As far as the survey of literature is concerned, no previous report on the structure activity relationship of various derivatives of cholesterol on the brain lipid profile is available following the administration of four derivatives of cholesterol A(I), B(II), C(III) and D(IV)[2a,b,c], injected ip 3.0 mg/kg body wt for 10 days in albino rats.



3β-Acetoxy-5-bromo- 6β-hydroxy 5α-cholestane (A)
(I)

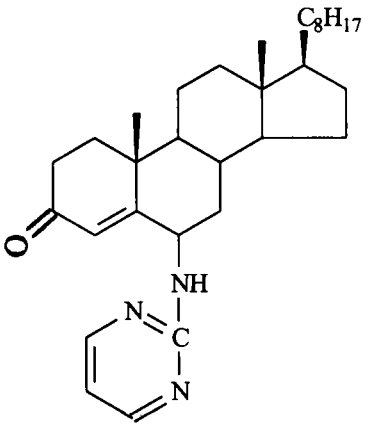


3β-Acetoxy-6-dimethylamino cholest-5-ene (B)
(II)



3β-Chloro-5-bromo, 6β-nitro-5 α-cholestane (C)

(III)



6β-Aminopyrimidino-cholest-4en-3one (D)
(IV)

Table – 3 (I)

Results :

Effects of steroidal derivatives on different regions of albino rat brain, injected 3.0 mg/kg body weight for 10 days, ip (n = 6)

Parameter	Brain region	Control (Average)	Experimental Structure – Activity – Correlation							
			A	% Change	B	% Change	C	% Change	D	% Char
TOTAL LIPIDS	Cerebrum	0.404	** 0.5521	-14.33	NS 0.5520	-5.67	*** 0.5518	110.2	*** 0.5522	359
	Cerebellum	0.544	*** 0.5830	-36.7	** 0.5835	-41.13	*** 0.5832	175.1	*** 0.5820	-66.
	Brain Stem	0.423	*** 0.420	89.7	*** 0.428	174.1	*** 0.435	209.7	*** 0.415	165

C = Control; % = Percentage

A = 3β-Acetoxy-5-bromo-6β-hydroxy-5α-cholestane (I)

B = 3β-Acetoxy-6-dimethylamino cholest-5-ene (II)

C = 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (III)

D = 6β-Aminopyrimidino-cholest-4-en-3-one (IV)

Values : *P<0.05; **P<0.01; ***P<0.001

NS = Not significant

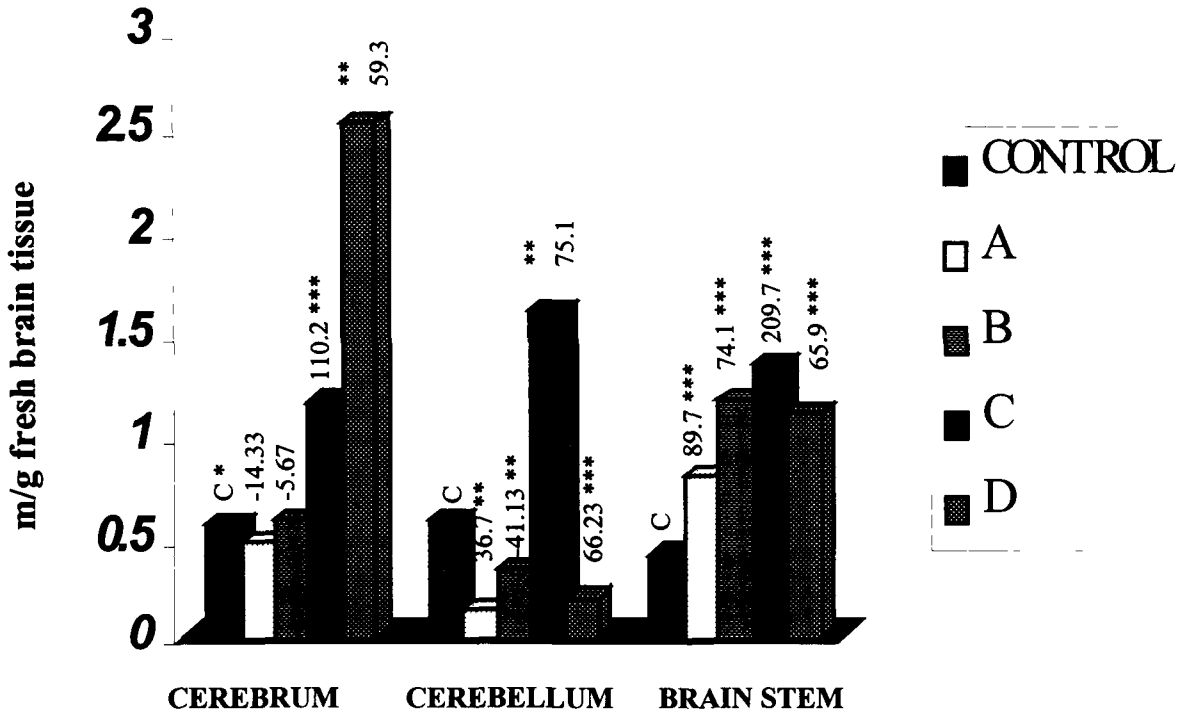
n = No of rats.

Table – 3 (I) shows the effects of four steroidal derivatives (3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane (A) (I); 3β-Acetoxy-6-dimethylamino-cholest-5-ene (B) (II); 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (C) (III); 6β-Aminopyrimidino-cholest-4-en-3-one (D) (IV) on the total lipids concentration in rats brain. The 3β-Chloro-5-bromo (C) (III) produces significant increase of 1102% in the cerebrum and 175.1% in the cerebellum, while most significant increase of 209.7% in the total lipid contents is observed in brain stem.

The 6β-Aminopyrimidino-cholest-4-en-3-one (D) (IV) produced significant 165.9% increase an most signifiicant increase in the total lipids contents of Brain stem and of cereberum 359.3% is observed, while significant decrease –66.23% occurs in brain stem.

The 3β-Acetoxy-6-dimethylamino-cholest5-ene (B) (II) produces significant increase 174.1% and significant decrease of –41.13% in the concentration of total lipids in brain stem and cerebellum respectively and of insignificant –5.67% decrease in cerebellum.

The 3β-acetoxy-5-bromo-6β-hydroxy-5α-(A) (I) produced significant decrease of -36.7% and significant increase 89.7% in the concentration of total lipids in cerebellum and cerebrum and it shows insignificant decrease of – 14.33% in brain stem respectively.



(Fig. 1)

Regional alteration in the level of total lipids in different regions of rat brain following the administration of 3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane (A) (I), 3β-Acetoxy-6-dimethylamino-cholest-5-ene (B) (II), 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (C) (III) and 6β-Aminopyrimidino-chlolest-4-en-3-one (D) (IV), 3 mg/kg body weight ip for 10 days.

In the comparative study of the effects of four steroidal compounds on major regions of rat brain (cerebrum, cerebellum and brain stem), the total lipids contents were found significant increase with the administration of C (III) derivative on cerebrum, cerebellum and brain stem of rat brain respectively and induced by compound D (IV) where most significant increase is 359.3% on cerebrum. In the Brain stem gradual significant rise was observed following the administration of A (I), B (II), D (IV)and C (III) compounds respectively. 209.7% Maximum significant rise was observed with the C (III) derivative, 359.3% significant increase was observed with the D (IV) compound and 174.1% significant increase was observed with the B (II) compound and 89.7% significant decrease was observed with the compound A (I). In cerebellum administration of A (I) and B (II) derivatives of cholesterol result in the significantly and gradual decrease in total lipids contents 41.13% and 36.7%, with the C (III) and D (IV) derivatives a significant increase was observed of 175.1% and decrease of –66.23% respectively. In brain stem administration of compound A (I) and B (II) derivatives of cholesterol results

in significantly and gradual increase in total lipids contents 89.7% and 174.1%, with the C (III) and D (IV) derivatives of cholesterol produced significant increase of 209.7%, 165.9% was estimated respectively.

Activity of 3β-acetoxy-5-bromo-6β-hydroxy (A) (I) in the three brain parts gives the trend of decreased and increased values of total lipids contents is in the order : cerebrum (-14.33%)< cerebellum (-36.7%)< brain stem (89.7%).

Activity of 3β-Acetoxy-6-dimethylamino (B) (II) gives the decreased and increased values of total lipids contents in this order : cerebrum (-5.67%) < cerebellum (-41.13%) < brain stem (174.1%). Activity of 3β-Chloro-5-bromo (C) (III) gives the rising trend of total lipids contents : cerebrum (110.2%) > cerebellum (175.1%) > Brain stem (209.7%). Activity of 6β-Aminopyrimidino (D) (IV) gives the rising trend as : cerebellum (-66.23%) > Brain stem (165.9%) > cerebrum (359.3%).

Changes in the Lipids contents and lipids composition must be due to changes in rates of anabolism, catabolism or these processes are controlled by the activities of appropriate enzymes. Hazzard and co-workers (1969) have suggested that a reduced lipoprotein lipase activity may be a factor contributing to the increased plasma lipids.

Depletion of unsaturated lipids is associated with alteration in membrane fluidity[69] and the changes in the activity of membrane bound enzymes and receptors[70]. Polyunsaturated fatty acids are by themselves, capable of causing damages to the cell membranes, tissues and may contribute to the postraumatic decline in the blood flow as well as reactive unflammatory responses. Interestingly lipids of various tissues are known to be in a dynamic steady state and there is continuous replacement of existing molecules by new ones[71]. Present study reveals the effects of four different derivatives of cholesterol on lipids contents of various parts of brain, is different on each part. Earlier it was concluded that estrogen affects the lipids contents differentially in the various parts of the brain.

CHOLESTEROL :

Cholesterol is a major and only sterol, present in the significant amount in the central nervous system[72], it is also an important component of biological membranes. Membranes are generally thought to consist of phospholipid bilayer into which membrane proteins are embedded, yet cholesterol molecules are present in most animal structures. Due to its amphipathic nature bearing an OH-group and a hydrocarbon skeleton with rigid rings and a branched chain of eight carbon, cholesterol is perfectly suited to mesh with

lipid bilayer[44]. Cholesterol accounts for about 10% of dry weight of the brain in contrast to less than 1% found in most other organs. The constancy of the amount of cholesterol in the brain suggests that the sterol is metabolically stable[73]. Unesterified cholesterol has been suggested as a lipid characteristic of myelin sheath, as it occurs in white matter in a higher concentration than in gray matter[74]. About 25% of cholesterol is present in myelin lipid by weight[75] and approximately 70% of total brain, cholesterol is present in myelin[76]. Cholesterol is thought to act as convenor in absorption of fats. It has been[77] reported a parallelism between cholesterol content of blood and the fatty acids. Due to abundance of cholesterol in nervous tissues and its variation in mental diseases, it may function as an insulating medium for myelin sheaths. Sterols are thought to have a role in maintaining the balance between the cell permeability and the membrane equilibrium of living cells. Brain microsomes are the sites of cholesterol biosynthesis[78]. Cholesterol is synthesized from acetate and precursors via mevalonic acid. Both the biosynthesis and the deposition of cholesterol in the CNS is most rapid. Biosynthesis of cholesterol in brain is most rapid during active myelination, but adult brain retains the capacity to synthesize cholesterol when precursors such as acetate or mevalonate are available.

Fatal or neonatal brain prior to myelination contains relatively little cholesterol. It has been found[79] varying amounts of the sterol in new born rat

brain. In rat brain, total levels of sterol ester increase from birth to 40 days. A decline is also noticed in human and guinea pig brain cholesterol (62.3%) and denosterol (31.1%). The major sterol and small to trace amount of other sterols were also detected[80] relative to total phospholipid and glycolipid, in which changes in whole brain and myelin are compared. Earlier studies in our laboratory on the alteration in cholesterol level following the intramuscular administration of ethynylestradiol and 0.5 mg norgestrol on the regional lipids level of cholesterol was found decreased in hypothalamus, hippocampus, amygdaloid nucleus midline nuclei of thalamus and gyrus cinguli[68]. It has been observed a depleted level of cholesterol in brain stem and spinal cord but it was elevated in cerebellum following the ip administration of estrogen in female rabbit.

TABLE – 3 (II)

Results :

Effects of steroidal derivatives on different regions of albino rat brain, injected. 3.0 mg/kg body weight for 10 days ip, (n=6)

Parameter	Brain region	Control (Average)	Experimental Structure – Activity – Correlation							
			A	% Change	B	% Change	C	% Change	D	% Chang
CHOLESTEROL	Cerebrum	0.0149	*** 0.0148	-81.8	*** 0.0152	-89.5	*** 0.0150	-57.3	*** 0.0146	-119.2
	Cerebellum	0.0023	*** 0.0023	-73.9	*** 0.0027	-51.9	*** 0.0021	-361.9	*** 0.0020	460.0
	Brain Stem	0.0089	*** 0.0088	-32.95	*** 0.0090	-57.8	NS 0.0085	-14.12	NS 0.0092	14.13

C = Control; % = Percentage

A = 3β-Acetoxy-5-bromo-6β-hydroxy- -5α-cholestane (I)

B = 3β-Acetoxy-6-dimethylamino-cholest-5-ene (II)

C = 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (III)

D = 6β-Aminopyrimidino-cholest-4-en-3-one (IV)

Values : *P<0.05; **P<0.01; ***P<0.001

NS = Not significant

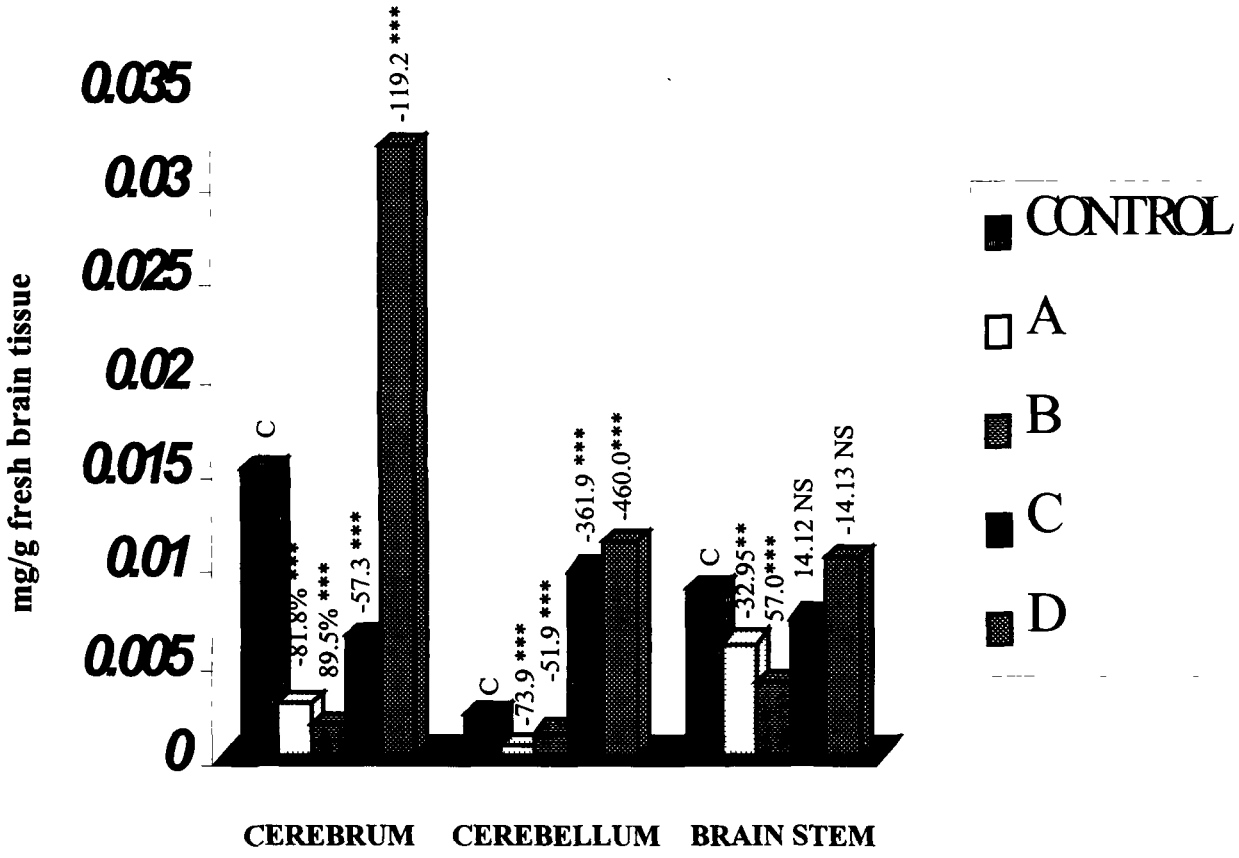
n = No of rats.

Table – 3 (II) shows that 6β-Aminopyrimidino (D) (IV) produces most significant increase 460.0% in cerebellum, while significant decrease is observed –119.2% of cerebrum and 14.13% in brain stem.

3β-Chloro-5-bromo-6β (C) (III) produces significant decrease –361.9% in the concentration of cholesterol in cerebellum and –57.3% in cerebrum, while it produces an insignificant deprivation in brain stem (-14.12%).

3β-Acetoxy-5-bromo (B) (II) produces significant decrease -89.7% in cerebrum, -57.8% in brain stem and –51.9% in the concentration of cholesterol in cerebellum.

3β-Acetoxy-5-bromo-6β-hydroxy (A) (I) produces significant decrease in the cholesterol level in cerebrum (-81.8%) in cerebellum (-73.9%) and in brain stem (-32.9%).



(Fig. 2)

Regional alteration in the levels of cholesterol following the administration of 3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane (A) (I), 3β-Acetoxy-6-dimethylamino-cholest-5-ene (B) (II), 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (C) (III) and 6β-Aminopyrimidino-cholest-4-en-3-one (D) (IV), 3 mg/kg body weight ip for 10 days in different regions of rat brain.

In the present study four derivatives of cholesterol 3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane (A) (I), 3β-Acetoxy-6-dimethylamino-cholest-5-ene (B) (II), 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (C) (III), 6β-Aminopyrimidino-cholest-4-en-3-one (D) (IV) were injected in the 4 groups of ablino rats (3 mg/kg body wt ip. for 10 days), changes in the cholesterol level estimated in the cerebrum, cerebellum and brain stem.

However no systematic work on structure activity correlation is done so far on the various homologous of cholesterol on the brain. Earlier Islam et. al.,[68] has studied the effect of estrogen and primovlar (having slight difference in the structure) on cholesterol level in the estrogen treated rabbits. It shows regional heterogeneity in exhibiting a depletion in the brain stem, spinal cord and increase in the cholesterol level in the cerebellum following 30 days administration. Primovlar treated rabbits shows the depletion of cholesterol level in hippo campus, amygdaloid nucleus, midline nuclei of the thalamus and gyrus cinguli in his study Islam also noted that alteration of cholesterol level was only significant after 90 days administration.

In the present study effect of the four derivatives were evaluated in the brain of albino rats, activity of these steroids in the cerebrum is as following. Four of these steroidal derivatives A (I), B (II), C (III) and D (IV) gives significant decrease in the cholesterol level, among these B (II) gives –89.5%,

C (III) –57.3%, A (I) –81.8% and D (IV) –119.2% in cerebrum. In the cerebellum compound D (IV) gives the most significant increase of 460.0% while compound B (II) and A (I) give significant decrease of –51.9% and 73.9% respectively, whereas compound C (III) gives 361.9% significant decrease in cholesterol level.

In the brain stem activity of compound B (II) and A (I) gives significant decrease in cholesterol level -57.8% and –32.95% while compound C (III) and D (IV) gives the insignificant decrease and increase of –14.12% and 14.13% respectively in cholesterol level.

Analysis of the effects of compound A (I) on all the three brain parts reveals its maximum effect on cerebrum (-81.8%) than cerebellum (-73.9%) a significant decrease and less effective on brain stem is of –32.95%. Analysis of the effects of compound B (II) on different parts of the brain reveals its maximum effect on cerebrum (-89.5%) than brain stem (-57.8%) and then in cerebellum (-51.9%), its shows significant decrease on all parameters. The activities of compound C (III) on these three brain parts reveal its most significant decrease of–361.9%on cerebellum and then in cerebrum (-57.3%) is of significant decrease, whereas insignificant decrease in the cholesterol level in cerebellum (-14.12%) is reported in this study.

Analysis of the effects of compound D (IV) on three brain parts reveals its most significant increase of 460.0% on cerebellum, where as significant

decrease on cerebrum (-119.2%) and on brain stem (14.13%) in the cholesterol level is reported.

A comparative study related to the activity of all four derivatives reveals that steroidal derivative (compound) D (IV) exerts its most significant rise on cerebellum (460.0%) and induced depletion in the cholesterol level. Compound C (III) induces most significant decrease on cerebellum of 361.9% and also gives significant depletion in cerebrum and insignificant depletion on brain stem of –14.12% is reported. Compound B (II) induces maximum significant decrease in cerebrum, cerebellum and brain stem, compound A (I) induces maximum depletion on cerebrum, cerebellum and brain stem respectively.

GANGLIOSIDES :

Gangliosides are the sialic acid containing glycosphingolipids, which are highly enriched in the CNS of vertebrate, including man[81,82,83,84]. Sialic acid is the generic name for N-acylneuraminic acid, the acyl group of sialic acid in the human brain is always the acetyl form[3]. N-acetyl neuraminic acid is commonly abbreviated as Neu Nac. Gangliosides are localized in two fractions of brain. Major gangliosides of the brain are GM1, GM2, GM3, GD1a, GD1b and GT and other minors are GD3, GD2 and sialylagal

actosylceramide are also present. Gangliosides under go characteristic changes in content and composition during development[86]. In particular they appears to be functionally involved in the control of axonal[87,88] out growth synaptogenesis and the establishment of cell contact. More over in adult nervous system, the individual gangliosides have been suggested to play a role as membrane bound receptors or co-receptors for toxins, drugs viruses hormones transmitters etc.[83]. Gangliosides are generally synthesized in neuronal perikarya and are transported to the nerve ending along with macromolecules[89,90,91]. Local synthesis within the axons and nerve ending[90] or even at the plasma membrane level[92] can not be excluded. Gangliosides are involved in nerve impulse conduction since they act as receptor sites for neurotoxins[93,94]. Irwin and Samson[95] reported the certain types of behavioural stimulations (stress, sensory stimulation, learning exercise) seems to be accompanied by alterations in gangliosides metabolism. Mental retardation and neurological dysfunction are major signs in nearly all the lipid storage diseases, apparently because of abnormal deposition of the different glycosphingolipids in the CNS due to defective disorders in degradative enzyme pathways, resulting in retardation of development paralysis dementia and blindness[60]. Biosynthesis of brain gangliosides occurs by sequential addition of monosacharides or N-acetylneuraminic acid to the carbohydrate

chain, starting from ceramide degradation of brain gangliosides. It proceeds by sequential removal of monosacharides and Neu-Nac (N-acetyl neuraminic acid) by glycosides and neuraminidases[96,97]. Gangliosides are responsible for the humeral immune response[98]. Evidences accumulated throughout the 1980s indicate the humeral immunity to gangliosides is frequently neuropathies[99], for example guillain barre syndrome[98]. The gangliosides mixture of bovine brain gangliosides (GM1, GD1a, GD1b, GT1b) has been widely used in trials of the treatment of peripheral neuropathies[100]. Gangliosides have been used for treatment of various degenerative toxic and metabolic neuropathies in Europe although their efficiency remains controversial[101]. Patients with multifocal motor neuropathy have high titer serum antibodies against the gangliosides GM1[85]. In lewis rats, myelin induced experimental allergic neuritis or animal model of human acute gullian barre syndrome can be depressed and delayed by adding gangliosides mixture (GM1, GD1a, GD1b, GT1b) to the immunization compound. However gangliosides may enhance the induction of adjuvant arthritis since externally applied gangliosides produce antibodies[102].

TABLE – 3 (III)

Results :

Effects of Steroidal derivatives on different regions of albino rat brain, injected 3.0 mg/kg body weight for 10 days ip. (n=6)

Parameter	Brain region	Control (Average)	Experimental Structure – Activity – Correlation							
			A	% Change	B	% Change	C	% Change	D	% Change
GANGLIOSIDES	Cerebrum	0.0024	** 0.0020	25.0	*** 0.0028	110.7	NS 0.0026	-7.69	*** 0.0024	337.5
	Cerebellum	0.00045	** 0.00043	-46.5	*** 0.00048	418.8	*** 0.00044	446.9	*** 0.00040	1157.5
	Brain Stem	0.00026	*** 0.00027	-59.3	*** 0.00024	700.0	*** 0.00030	780.0	*** 0.00023	2760.9

C = Control; % = Percentage

A = 3β-Acetoxy-5-bromo-6β-hydroxy-5α-cholestane (I)

B = 3β-Acetoxy-6-dimethylamino-cholest-5-ene (II)

C = 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (III)

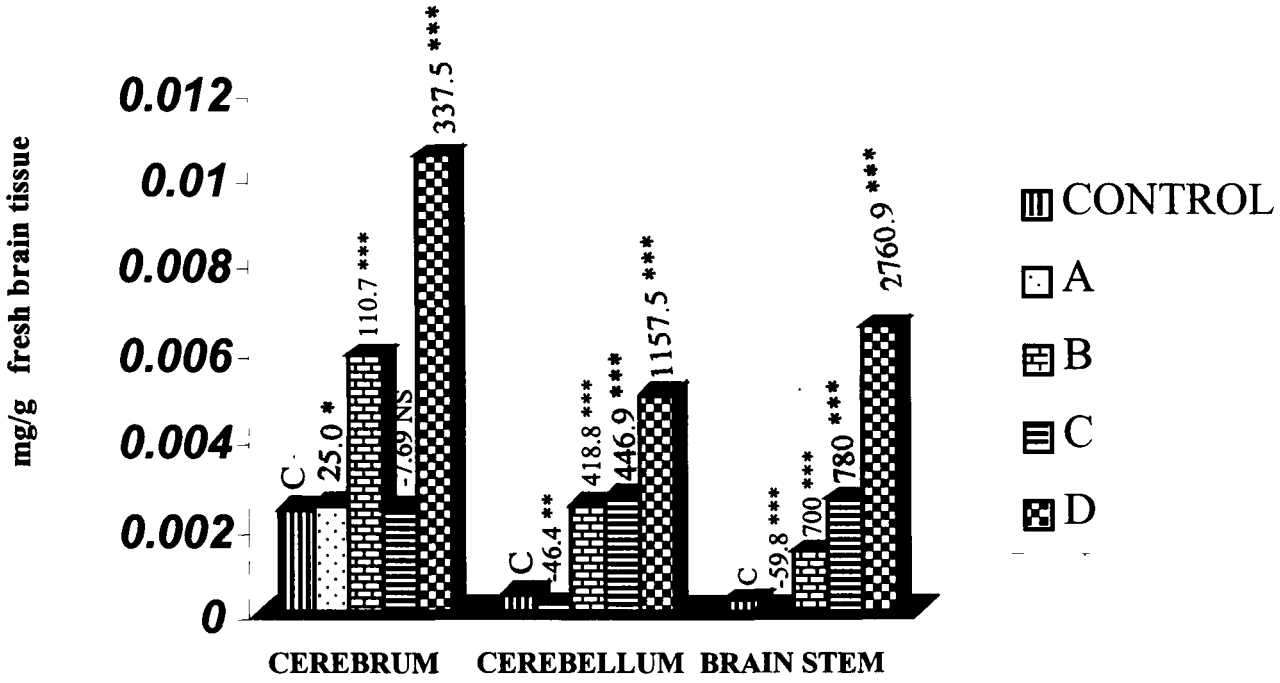
D = 6β-Aminopyrimidino-cholest-4-en-3-one (IV)

Values : *P<0.05; **P<0.01; ***P<0.001

NS = Not significant

n = No of rats.2

Table – 3 (III) shows that the 6β-Aminopyrimidino (D) (IV) produces most significant increase in the level of gangliosides in brain stem (2760.9%) and cerebellum (1157.5%) and cerebrum (337.5%). 3β-chloro-5-bromo (C) (III) produces most significant increase in level of gangliosides in brain stem (780.0%), cerebellum (446.9%) and insignificant decrease in cerebrun (-7.69%) is observed. 3β-Acetoxy-6-dimethylamino (B) (II) produces most significant increase in brain stem (700.0%), cerebellum (418.8%) and in cerebrum (110.7%). 3β-Acetoxy-5-bromo-6β (A) (I) produces significant decrease in level of gangliosides in brain stem (-59.3%) and in cerebellum (-46.5%). While significant increase is observed (25.0%) in cerebrum.



(Fig. 3)

Regional alteration in the levels of gangliosides in different regions of rat brain following the administration of 3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane (A) (I), 3β-Acetoxy-6-dimethylamino-cholest-5-ene (B) (II), 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (C) (III) and 6β-Aminopyrimidino-cholest-4-en-3-one (D) (IV), 3 mg/kg body weight ip, for 10 days.

Studies on gangliosides had been done earlier in our laboratory. Intramuscular administration of 100 μg estrogen daily for 30 days to female rabbit was followed by decrease in gangliosides in cerebellum and spinal cord while an elevated level was observed in cerebral cortex and brain stem[103].

There is no previous report on the classified study on structure activity correlation on the level of gangliosides in the brain while using four derivatives of cholesterol (A (I), B (II), C (III) and D (IV)). In the present study changes were found as following in the cerebrum, cerebellum and brain stem. When the activity of steroidal derivatives (compound A (I), B (II), C (III), D (IV)) was measured in the different brains parts of albino rat following the administration of 3.0 mg/kg body wt. of steroids for 10 days, significant rise in the gangliosides contents from the effect of compound D (IV) (337.5%) and compound B (II) (110.7%) where as insignificant decrease in gangliosides from the effect of compound C (III) (-7.69%) and compound A (I) (25.0%) a significant increase is estimated in cerebrum.

In the cerebellum, maximum and most significant increase with the administration of compound D (IV) (1157.5%), compound C (III) (446.9%) and compound B (II) (418.8%) in the contents of gangliosides is reported, where as significant decrease in content of gangliosides with compound A (I) (-46.5%) is reported.

Estimation of gangliosides in the brain stem shows most significant increase with the administration of compound D (IV) (2760.9%), compound C (III) (780.0%) and of compound B (II) (700.0%), while significant decrease in content of gangliosides with compound A (I) (-59.3%) is estimated.

Analysis of the effects of compound C (III) on three brain parts reveals its maximum and most significant increase on brain stem (780.0%) and on cerebellum (446.9%), while on cerebrum (-7.69%) in gangliosides content is reported.

Similarly analysis of the effects of compound D (IV) reveals it maximum and most significant increase on brain stem (2760.9%), cerebellum (1157.5%) and on cerebrum (337.5%) is estimated.

Effects of compound (B) (II) reveals it most significant increase on brain stem (7000%), cerebellum (418.8%) and cerebrum (110.7%) in content of gangliosides is reported.

In the analysis of effects of compound A (I) reveals its significant increase on cerebrum (25.0%) and significant decrease on brain stem (-59.3%) and on cerebellum (-46.5%) in the contents of gangliosides is estimated.

A comparative study of four steroidal derivatives reveals that compound D (IV) exerts its maximum and most significant increase on brain stem (2760.9%), cerebellum (1157.5%) and on cerebrum (337.5%) in the contents of gangliosides whereas compound C (III) also produces maximum and most significant rise on brain stem (780.0%), cerebellum (446.9%) while insignificant decrease of (-7.69%) on cerebrum is reported. While compound B (II) produce most significant increase in the contents of gangliosides on three

major parts of brain. Compound A (I) shows significant increase on cerebrum (25.0%) in the study of gangliosides level.

LIPID PEROXIDATION :

Brain contains large amounts of lipids that are rich in polyunsaturated fatty acids. The unsaturated bonds of membrane lipids can readily react with free radicals and under go peroxidation[104]. Lipid peroxidation has been identified as a basic deterioative reaction in the cellular mechanism[105]. It is an autocatalytic free radical process. Biomembrane and subcellular organelles are the major sites of lipid Peroxidation[106]. Quantitative studies of enzymatic inactivation by lipid peroxidation have shown that sulfhydryl enzymes are most susceptible to inactivation[107]. Each lipid peroxide is a free radical and once initiated the process of peroxidation can become auto catalytic as each lipid peroxide products. The lipid peroxides readily decompose to liberate highly reactive carbony fragment such as melondialdehyde[105] (MDA), X-3-carbondialdehyde is one of the final products of free radical chain reaction which takes place during lipid peroxidation[108]. The H_2O_2 and other reaction give O_2 species if not scavenged efficiently are known to give rise to potentially toxic intermediates, namely hydroxy radical (OH) and singlet

($O_2^{\cdot}$). These oxidants in the presence of metal ions, result in the formation of lipid peroxidation[109].

Gutteridge[110] has shown that melondialdehyde is the major species responsible for thiobarbituric acid reactive products (TBA-RS). Whether the substances undergoing free radical induced damage are poly unsaturated fatty acids, amino acids, carbohydrates or nucleic acids. Therefore, measurement of thiobarbituric acid substance is one of the evaluating the extent of the damage due to free radicals. However the best induced effect of free radical attack is that causing lipid peroxidation i.e. oxidation of a methylene bridge of unsaturated fatty acids, resulting in the formation of lipid peroxides and hydroperoxides finally leading to fragmentation of lipids. As biomembranes are rich in unsaturated fatty acid, such reaction may lead to the disintegration of membrane structure and finally to irreversible cell damage[111].

Initiation of lipid peroxidation in a membrane or free fatty acid is due to the attack of any species that has sufficiently reactivity to abstract a hydrogen atom. Since a hydrogen atom has only one electrons, this leaves behind an unpaired electron on the carbon atom. The carbon radical in a polyunsaturated fatty acid tend to be stabilized by a molecular rearrangement to produce a conjugated diene, which rapidly reacts with O_2 to give hydroperoxy radical. Hydroperoxy radicals abstract hydrogen atoms from

other lipid molecules and so continues the chain reaction of lipid peroxidation. The hydroperoxy radical combines with the hydrogen atom that it abstracts to give a lipid hydroperoxide[112].

LH = Fatty acid, LOOH = Lipid hydro peroxides,

L° = Lipid alkyl radical

LOO° = Lipid peroxy radicals.

Initiation :

$LH + O_2$ ▶ Free radicals, L° (1)

LOOH ▶ Free radicals, LOO° (2)

Propogation.

$L° + O_2$ ▶ LOO°

LOO° + LH ▶ LOOH + L°

Termination.

LOO° + LOO° ▶ $LOOL + O_2$

LOO° + L° ▶ LOOL

L° + L° ▶ LL

Oxygen free radical induced oxidative damage has been implicated in the aetiology of a number of diseases, including atherosclerosis, inflammation, cancer, and ischemia reperfusion[113,114,115]. Toxicity of uraemia is due atleast

in part to oxygen free radical mediated lipid peroxidation and other tissue damaging effects[116]. There is considerable evidence suggesting the involvement of free radicals and lipid peroxidation in atherogenesis[117].

TABLE – 3 (IV)

Results :

Effects of steroidal derivatives on different regions of albino rat brain, injected 3.0 mg/kg body weight 10 days ip, (n=6)

Parameter	Brain region	Control (Average)	Experimental Structure – Activity – Correlation							
			A	% Change	B	% Change	C	% Change	D	% Change
LIPID PEROXIDATION	Cerebrum	0.0065	** 0.0060	-30.0	NS 0.00468	17.65	** 0.0066	-25.8	NS 0.0063	-6.34
	Cerebellum	0.0070	** 0.0064	-39.1	NS 0.0075	6.67	** 0.0070	-40.0	** 0.0070	-38.6
	Brain Stem	0.0057	*** 0.0059	-62.7	NS 0.0060	10.0	NS 0.0056	-21.4	NS 0.0054	-14.8

C = Control; % = Percentage

A = 3β-Acetoxy-5-bromo-6β-hydroxy-5α-cholestane (I)

B = 3β-Acetoxy-6-dimethylamino-cholest-5-ene (II)

C = 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (III)

D = 6β-Aminopyrimidino-cholest-4-en-3-one (IV)

Values : *P<0.05; **P<0.01; ***P<0.001

NS = Not significant

n = No of rats.

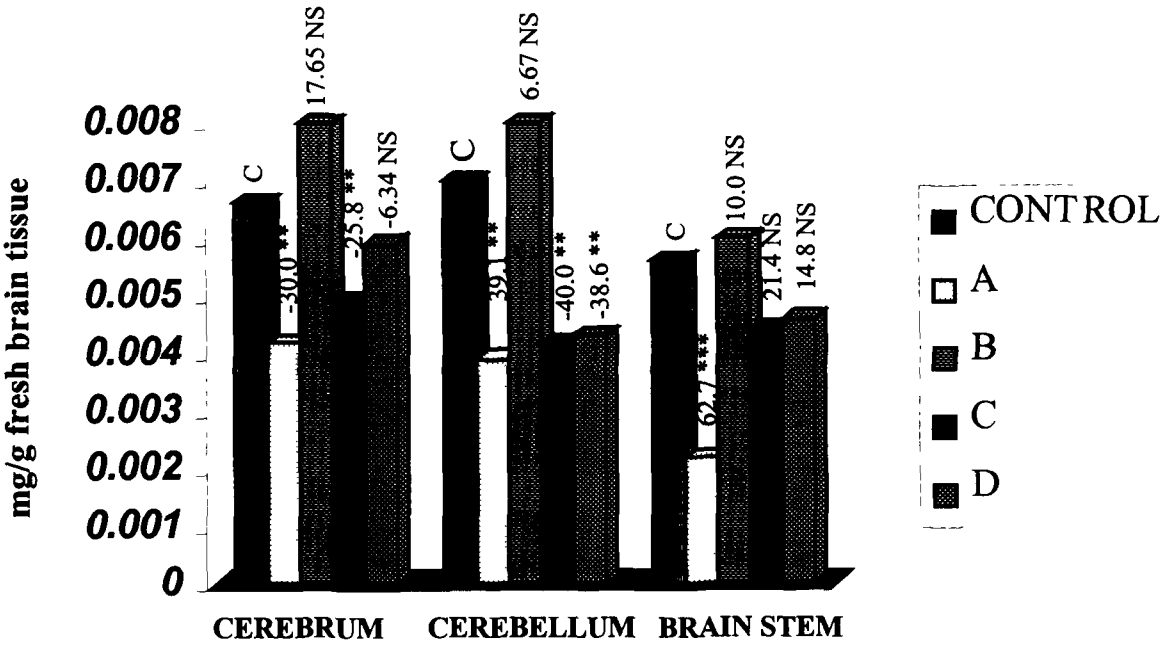
Table – 3 (IV) shows the data obtained for the rate of lipid Peroxidation following the administration of four steroidal derivatives.

3β-Acetoxy-5-bromo-6β-hydroxy (A) (I) produces significant decrease of 62.7% in brain stem, -39.1% in cerebellum and of cerebrum (-30.0%) is observed.

3β-Acetoxy-6-dimethylamino (B) (II) produces insignificant increase of 17.65% in cerebrum, 10.0% in brain stem and 6.67% is reported in cerebellum of rats brain in the study.

6β-Aminopyrimidino (D) (IV) produces significant decrease of –38.6% in cerebellum while insignificant depletion of 14.8% and 6.34% is found in brain stem and cerebrum respectively.

3β-Chloro-5-bromo (C) (III) produces significant decrease of 40.0% in cerebellum and of 25.8% in cerebrum. While insignificant decrease of 21.4% is reported in brain stem of rats brain in the study.



(Fig. 4)

Regional alteration in the level of rate of lipid peroxide formation in different regions of rat brain following the administration of 3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane (A) (I), 3β-Acetoxy-6-dimethylamino-cholest-5-ene (B) (II), 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (C) (III), 6β-Aminopyrimidino-cholest-4-en-3-one (D) (IV), 3.0 mg/kg body weight ip for 10 days.

In the present study, following the administration of compounds A (I), B (II), C (III) and D (IV), derivatives of cholesterol where rate of lipid peroxidation was estimated. Compound A (I) gives significant decrease (-30.0%) in the rate of lipid peroxidation, whereas compounds B (II) and D

(IV) gives relatively insignificant increase and decrease of 17.65% and –6.34% respectively, while compound C (III) gives significant depletion (-25.8%) in cerebrum of rats brain. Effects of these four derivatives on cerebellum shows significant depletion in the rate of lipid peroxidation as D (IV) (-38.6%), A (I) (-39.1%) and C (III) (-40.0%). Whereas compounds B (II) result in an insignificant rise (6.67%) in the rate of lipid peroxidation. In brain stem compounds A (I) give significant decrease of (-62.70%), where as compound B (II), C (III) and D (IV) gives insignificant enhancement and decrease of (10.0%), (-14.8%) and (-21.4%) in the rate of lipid peroxidation.

A comparative study of effects of steroidal compounds on different part of brain, in which compound A (I) gives most significant decrease on brain stem, cerebellum and cerebrum respectively, where as compound B (II) shows insignificant increase in cerebellum, brain stem and cerebrum respectively. Compound C (III) exhibits significant decrease on cerebellum, cerebrum and brain stem respectively. Whereas compound D (IV) exhibits significant decrease in cerebellum and insignificant decrease on brain stem and cerebrum in rate of lipid peroxidation of rat brain. On a comparative study we found to decrease the content of lipid peroxidation and a tool for lowering the aging.

EXPERIMENTAL

All the chemicals used in this study were of analytical reagent grade. We used the instruments in this study are DU-6 Beckman spectrophotometer, metabolic shaker (NSW, India), Digestor (Designed in our centre), homogenizer (Yorco scientific industries). Albino rats of charles foster strain (200±20 grams) obtained from animal house, J. N. Medical College, A.M.U., Aligarh. The steroidal compounds, 3β-acetoxy-5-bromo-6β-hydroxy-5α-cholestane A (I), 3β-Acetoxy-6-dimethylaminocholest-5-ene B (II), 3β-Chloro-5-bromo, 6β-nitro-5α-cholestane (C) (III) and 6β-Aminopyrimidino-cholest-4-en-3-one D (IV) were prepared according to literature procedures[2a,b,c].

Healthy male albino rats (Fig.5) of Charles Foster Strain, obtained from the animal house of J. N. Medical College, A.M.U., Aligarh were used in the present study. They were divided into four groups.

Group 1;6 rats wt. = 200 ± 20 g

Group 2;6 rats wt. = 200 ± 20 g

Group 3;3 rats wt. = 200 ± 20 g

Group 4;3 rats wt. = 200 ± 20 g

Albino Rat
Fig. 5

Solution Preparation And Dose Administration :

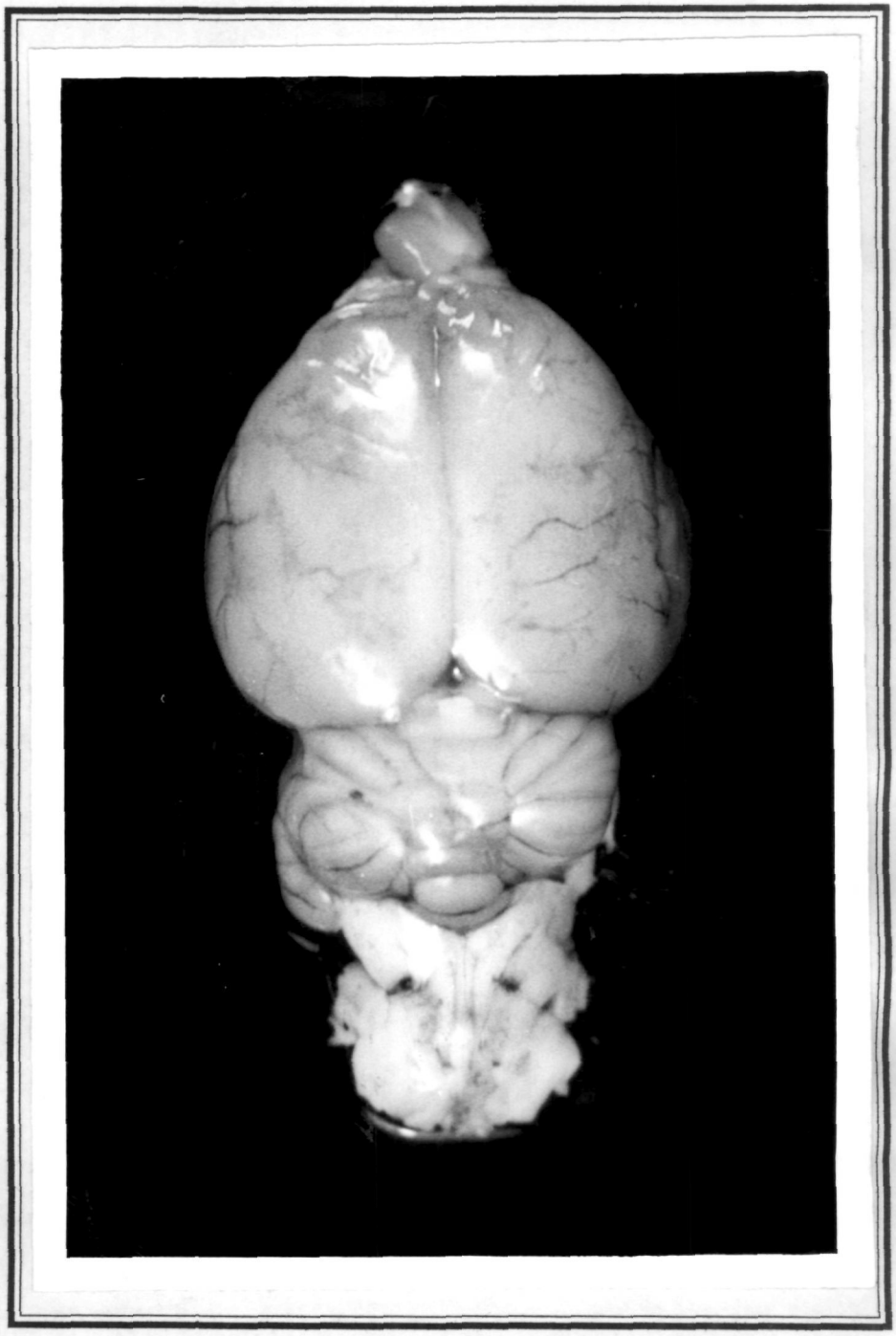
3.0 mg /kg body weight steroids were given to the rats. The solution for injection to the rats were prepared in a peanut oil and was made keeping in mind that the amount of the oil to be injected to the animal should not exceed approximately 0.5ml,to avoid the accumulation of fat (lipids) in albino rats. The solution was prepared by adding 240 mg reference steroids in 20ml of peanut oil .The steroids were injected intraperitoneally with the help of 2ml tuberculin glass syringe and 26 number needle. A new needle for each animal was used daily .The syringe were sterilized each day by boiling in water and rinsing with methanol.

Group 1: (Control) of 3 male albino rats were injected normal saline equal to the volume of steroidal solution for 10 days.

Group 1: (Experimental) of 3 male albino rats were injected 3β-Acetoxy-5-bromo-6β-hydroxy-5α-cholestane (A) (I), 3.0 mg /kg body weight for 10 days.

Group 2: (Control) of 3 male albino rats were injected normal saline equal to the volume of steroidal solution for 10 days.

Group 2: (Experimental) of 3 male albino rats were injected 3β-Acetoxy-6-dimethyl amino cholest-5-ene (B) (II), 3.0 mg/kg body weight for 10 days.



Photograph Showing the Dorsal View of Rat Brain
Fig. 6

Group 3: (Experimental) of 3 male albino rats were injected 3β-Chloro-5-bromo-6β-nitro-5α-cholestane (C) (III), 3.0 mg/kg body weight for 10 days.

Group 4: (Experimental) of 3 male albino rats were injected 6β-Aminopyrimidino-cholest-4-en-3-one (D) (IV), 3.0 mg/kg body weight for 10 days.

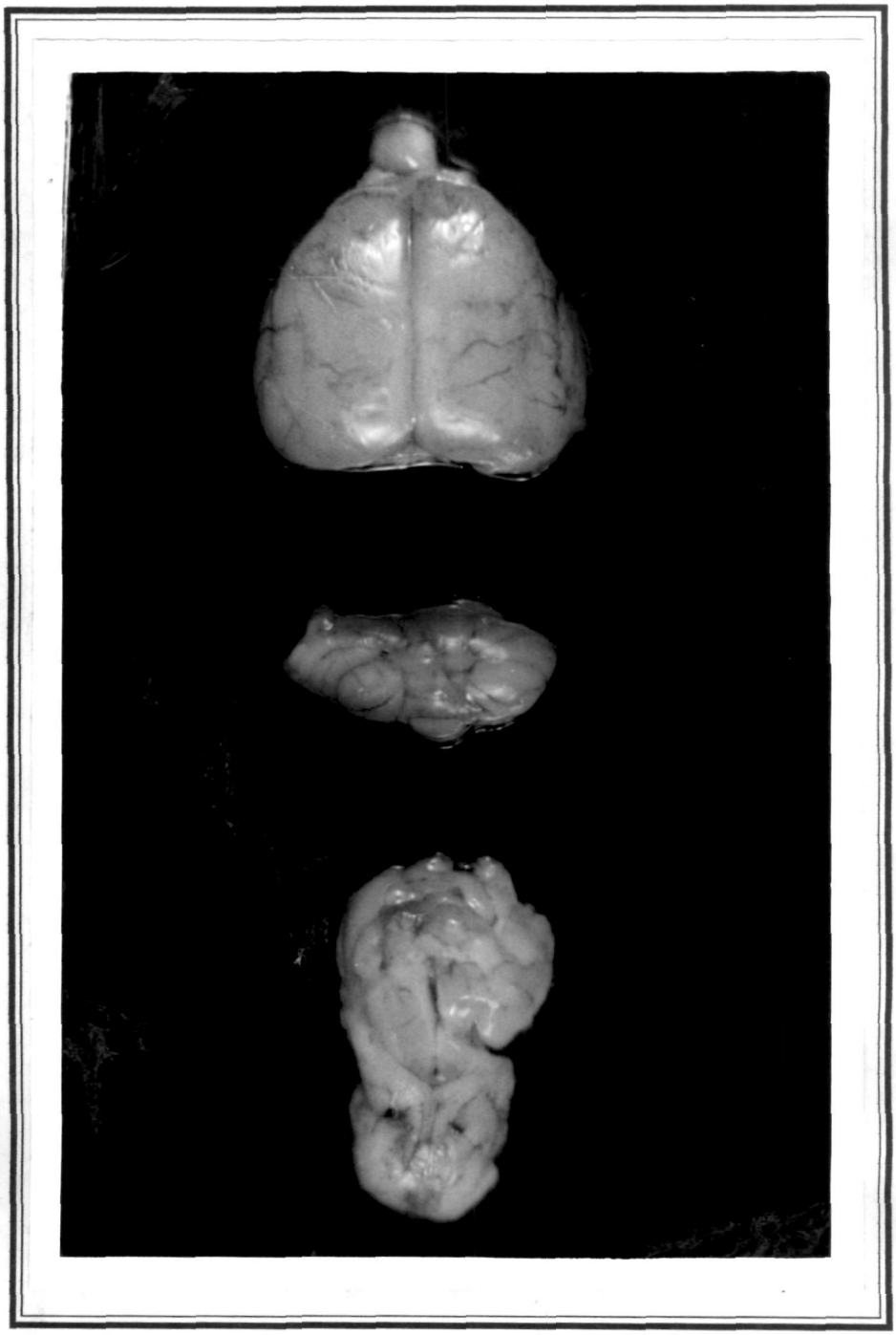
Dissection Of Different Parts Of The Rat Brain :

For all biochemical investigations, fresh unfixed brain (Fig. 6) was used .The brain and spinal cord were removed rapidly from individual rats and dissected out on an ice plate. The blood clots adhering to brain were removed by washing with cold normal saline. There after, the cerebrum, cerebellum, and brain stem (Fig. 7) were rapidly dissected out and weighed to the nearest milligram on an electrical balance were pooled and used for biochemical analysis.

For biochemical and most of the histochemical studies where perfusion of the rat brain was not required the animal were sacrificed.

Exposure Of The Brain And Its Removal :

The skeletal covering over the skull were removed with the use of a small pair of bone forceps, scalpel scissors and nail clippers. Great care was



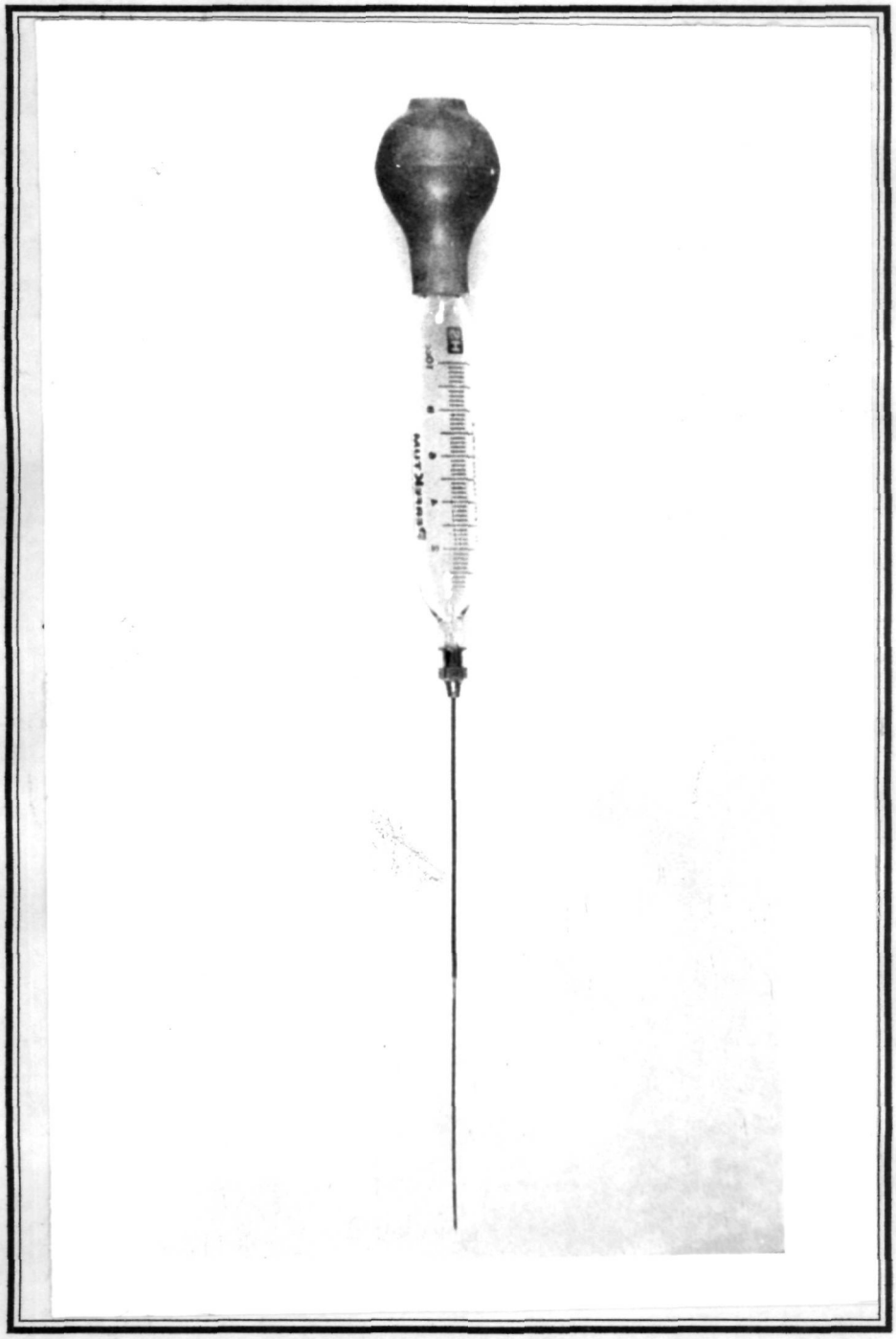
Dissection of Different Parts of Rat Brain
Fig. 7

taken to avoid any laceration of brain tissue. After exposing the brain surface from the top and sides, it was gently detached from the base of skull and removed.

Extraction And Purification Of Brain Lipids :

Lipids from brain region were extracted and purified immediately after dissection of the animal by the method of Fotch et. al.,[118]. This method was partially modified in our laboratory[68] for isolation of lipids from discrete areas of the brain. Different parts of the brain were weighed and homogenized (10% w/v) in a glass homogenizer to a final volume of 6 ml chloroform : methanol mixture (2:1, v/v). Each homogenate was shaken periodically for an hour and filtered through sintered glass funnel (G-4) under vacuum (Fig. 8). The residue of each test tube was again homogenized with 2 ml chloroform : methanol mixture and filtered. The resultant residue was washed several times with the solvent mixture to ensure complete extraction of lipids. The final volume of each extract was made upto 10 ml with chloroform-methanol mixture in a graduate test tube. Thereafter 2.5 ml of 0.9% NaCl was added to the extract in each test tube. This was shaken vigorously on test tube for complete mixing and placed at -20°C in a deep freeze overnight for complete separation of the two layers. The junction of the layers of each test tube was marked. The upper aqueous layer was taken out with a specially

Sintered Glass Funnel for Filteration
Designed in IBRC, A.M.U., Aligarh.
Fig. 8



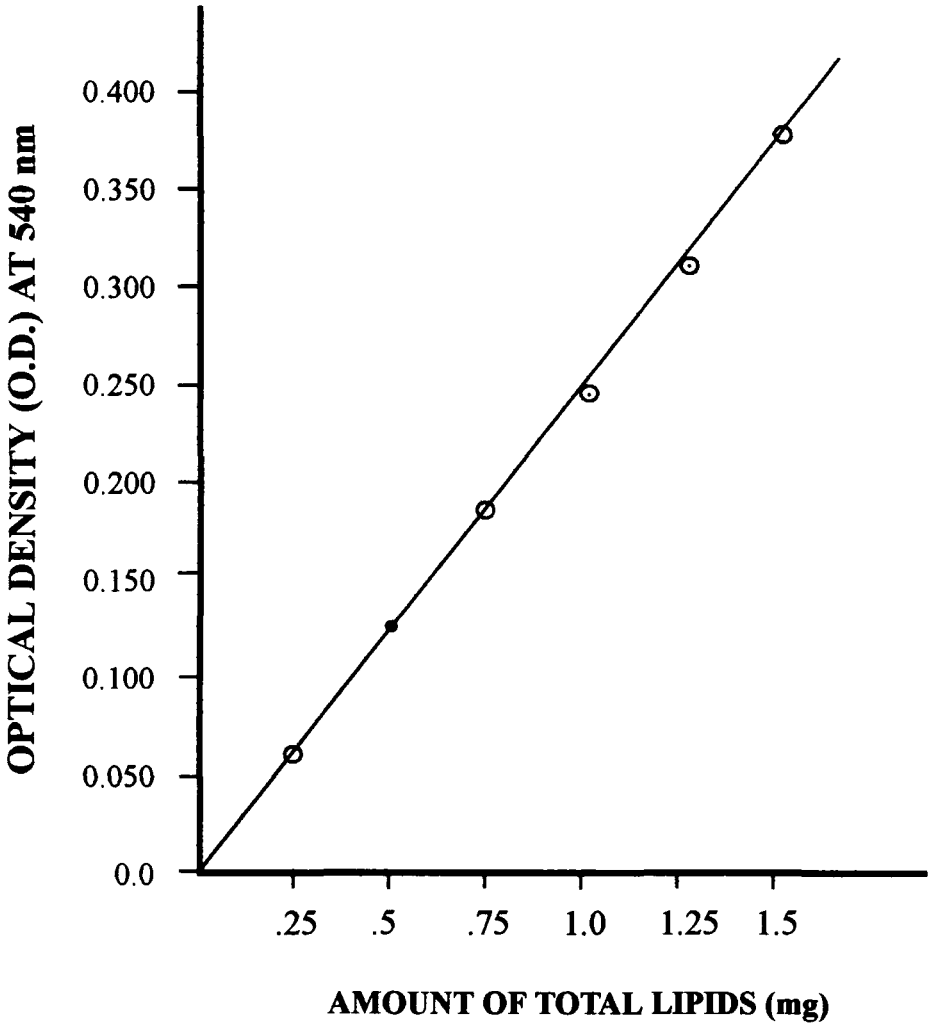
Specially Design Syringe
Designed in IBRC, A.M.U., Aligarh.
Fig. 9

designed syringe (Fig. 9) without disturbing the interfacial fluff. This upper layer was used for the estimation of gangliosides.

The lower lipid layer was made homogeneous by adding required amount of methanol, make the final volume to 10 ml and transferred to round bottom flask and dried under vacuum at 40 °C. In order to remove proteins, the residue was dissolved in chloroform : methanol : water mixture (64:32:4 v/v) and evaporated to dryness. The contents were suspended in chloroform : methanol (2:1 v/v) mixture and evaporated to dryness. The process was repeated again untill moisture was completely removed from lipids. The lipids were then suspended in chloroform, filtered free of any protein and filtrate was dried under vacuum at 40°C. The dried lipids were solubilized to a known volume with chloroform and stored at-20°C till further use. This extract was used for the estimation of total lipids, triglycerides and cholesterol.

Estimation Of Total Lipids : Total Lipids were estimated according to the method of woodman and price[119].

Colour was developed with the help of colouring reagent (phospho–vanillin) in the presence of sulphuric acid (H_2SO_4) and absorbance was read at 540 nm. The details of the reaction are given below. Sulphuric acid acts upon the double bond on lipid to produce carbonium ion which simultaneously

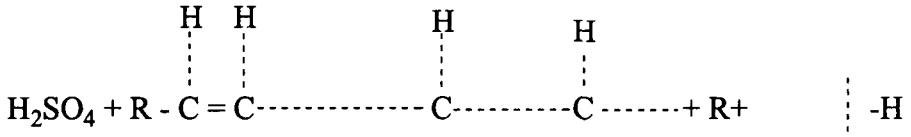


Standard Graph of Total Lipids

Method
Woodman and Price, (1972)

reacts with phosphate ester of vanillin to form coloured complex with an absorption maximum at 540 nm.

A standard solution of 0.5 mg brain lipid/ml in chloroform : methanol mixture (2:1 v/v) was prepared by diluting 1.0 ml of refrigerated stock solution (50 mg brain lipid / 10 ml) in chloroform : methanol mixture in a 10 ml volumetric flask and the volume was made upto mark with chloroform : methanol mixture.



6.0 Grams of potassium dihydrogen ortho Phosphate (KH_2PO_4) and 0.39 grams of vanillin were dissolved by heating in double distilled water and made up the volume to 100 ml in a volumetric flask (colouring reagent)

Lipids were isolated from the rat brain by the technique described in the extraction and purification of lipids.

0.1 ml of brain extract in duplicate was taken in 18 x 150 mm corning test tube. Conc. sulphuric acid (2.5 ml) was added to test tube and heated on boiling water bath for 20 minutes. After cooling 5.0 ml of colouring reagent was added and absorption was read at 540 nm after exactly 10 minutes in DU-6 Beckman spectrophotometer against a reagent blank. A calibration curve of absorbance in concentration (100 – 600 μg) of standard brain lipids was

prepared by adopting the same procedure as described above. The values of the standard curve were plotted by least square method. The absorbance of total lipids in brain samples were compared with curve and finally calculated by the following formula.

Calculation :

$$\textbf{Total Lipids (mg/g of fresh brain weight)} = \frac{\textbf{C x V}}{\textbf{Vt x Wt}}$$

Where

C = Concentration of lipids in μg in 0.1 ml extract.

V = Total volume of the total lipids extract

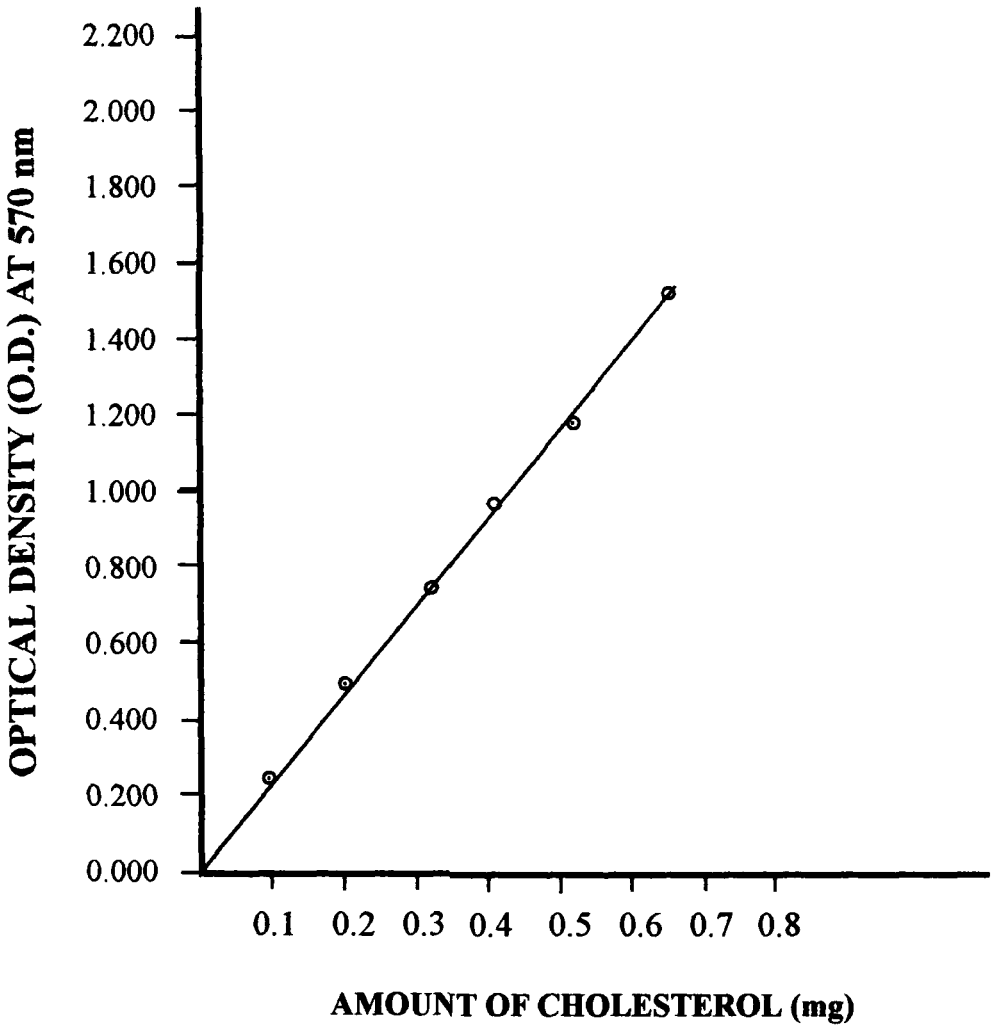
Vt = Volume taken for the estimation.

Wt = Fresh weight of the tissue in mg.

Estimation Of Cholesterol :

Total cholesterol was estimated according to the method of Zlatis et.al.[120].

Cholesterol dissolved with acetic acid in the presence of $FeCl_3 - H_2SO_4$ reagent gets dehydrogenated to 3.5 cholestadiene or 2, 3-cholestadiene which



Standard Graph of Cholesterol

Method
Zlatis et al. (1954)

simultaneously polymerizes and reacts with $FeCl_3$ to form a violet coloured complex which is measured clorimetrically at 570 nm.

Standard Solution :

A standard solution of 1.0 mg/ml cholesterol in acetic acid was prepared by diluting 1.0 ml of stock solution (100 mg cholesterol in 10 ml acetic acid) in 10 ml volumetric flask and the volume was made to 10 ml with acetic acid.

Stock Ferric Chloride Regent :

5.0 gm of anhydrous ferric chloride was dissolved in 50 ml of glacial acetic acid.

Working Ferric Chloride Reagent :

1.0 ml of stock ferric chloride was diluted to 100 ml with concentrated sulphuric acid.

Brain extract (0.05 ml) was taken in duplicate in dry 15 x 150 mm test tubes, dried and dissolved in 3 ml glacial acetic acid. Working Ferric Chloride solution (2 ml) was added and the contents were mixed thoroughly. The tubes were kept in dark for 30 minutes and the optical density was then measured at 570 nm. Reagent blank and standard cholesterol solution were also run simultaneously.

Calculation :

Standard curve of cholesterol was used to calculate the amount of cholesterol in the samples and results were expressed as mg/cholesterol/g tissue weight.

$$\textbf{Cholesterol (mg/g of fresh brain weight)} = \frac{C \times V}{Vt \times Wt}$$

Where

C = Concentration of cholesterol in μg in 2.0 ml extract.

V = Total volume of cholesterol extract

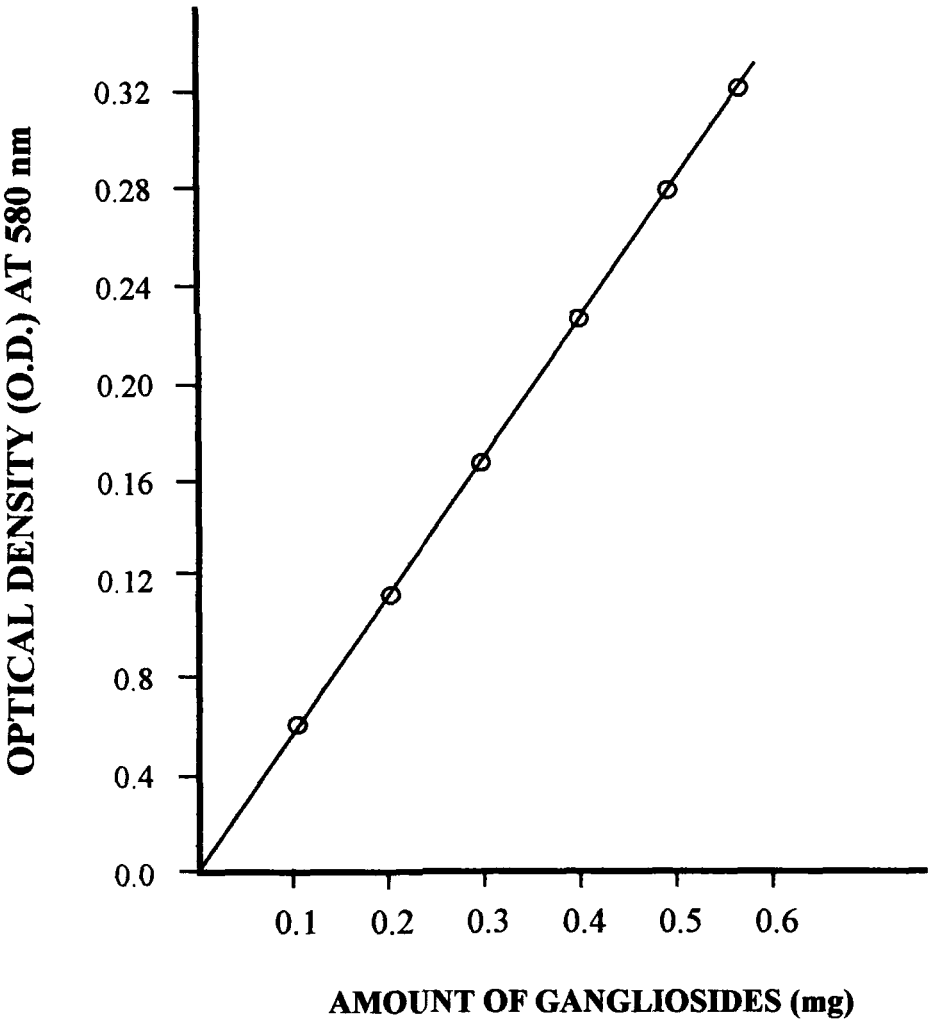
Vt = Volume taken for the estimation.

Wt = Fresh weight of the tissue in mg.

Estimation Of Gangliosides :

Gangliosides were estimated according to Pollet et. al.[121], after modification in the methods of Svennerholm (1980)[83], Miettinene and Takki-Luukkainen (1959).

Bound sialic acids in the gangliosides were isolated carefully without their degradations. When samples are heated at 100°C in a boiling water bath for 30 minutes in resorcinol reagent, the pentose sugar moieties break up and make a coloured complex with resorcinol reagent. This coloured complex is



Standard Graph of Gangliosides

Method
Pollet et al. (1978)
Modified by
Svennerholm, (1957), Miettinene and Takki - Luukkainen, (1959)

extracted in organic solvent (butyl acetate and n-butanol; 85:15, v/v). organic Phase was taken and absorbance was measured at 580 nm.

Standard Solution :

A stock standard was prepared by dissolving 100 mg N-acetylneuraminic acid in double distilled water and making the final volume to 100 ml. This gives 1.0 mg N-acetylneuraminic acid/ml. Working standard was prepared by taking 1.0 ml of stock standard solution and diluting it to 10 ml in a volumetric flask with double distilled water. This is 100 µg/ml of N-acetylneuraminic acid/ml.

Resorcinol Reagent :

This reagent was prepared by mixing 10 ml of 3.0% resorcinol solution in double distilled water, 80 ml of concentrated HCl, 0.25 ml of 0.1 N copper sulphate and double distilled water upto 100 ml mark in a volumetric flark.

Resorcinol reagent (2 ml) was added to 2.0 ml of the upper layer (aqueous) of lipid extract and heated in a boiling water bath for 30 min. After cooling to room temperature, 5.0 ml of mixture of butyl acetate and n-butanol (85:15, v/v) was added to each tube. The tubes were shaken thoroughly and kept for 15 minutes to separate organic phase. About 3-4 ml of organic phase

was taken and absorbance was measured 580 nm against a reagent blank. A standard curve with absorbance of different concentrations of n-acetylneuraminic acid (5-30 μg) having 2 ml final volume was prepared by treating in the same way as samples. The absorbance of test samples were compared with those of standard curve and gangliosides were quantitated as below.

Calculation :

$$\textbf{Gangliosides (mg/g of fresh brain weight)} = \frac{\textbf{C x V}}{\textbf{Vt x Wt}}$$

Where

C = Concentration of gangliosides in mg in 2.0 ml extract.

V = Total volume of the upper layer extract

Vt = Volume taken for the estimation.

Wt = Fresh weight of the brain tissue in mg.

Determination Of The Rate Of Lipid Peroxidation :

The procedure of Utely et. al.[122], was used for finding out the rate of lipid peroxidation.

0.15 M Potassium Chloride :

This solution was prepared by dissolving 2.8 gm KCl in 250 ml double distilled water.

10% Trichloroacetic Acid (TCA) :

10% TCA was prepared by dissolving 10 gm TCA in 100 ml distilled water.

0.67% Thiobarbituric Acid (TBA) :

Thiobarbituric acid 0.67 gm was dissolved in 25 to 50 ml double distilled water by adding two pellets of NaOH. The pH of the solution was adjusted to 7.2 with the help of 1N HCl and volume was made upto 100 ml with double distilled water.

Different parts of the brain were homogenized (10% v/v) in chilled 0.15 M KCl and then Brain extract 0.5 ml with 3.6 ml TCA was taken, precipitate comes thereafter homogenate was pipetted in centrifuge tube and protein was precipitated because of addition of 10% TCA and the reaction mixture was centrifuged at 5000 rpm for 10 minutes. Two ml of clear supernatant was mixed with 1.5 ml of 0.67% TBA and 1.0 ml of double distilled water, placed in a boiling water bath for 10 minutes cooled and the absorbance of the colour was read at 532 nm. The rate of lipid peroxidation

was expressed as nanomoles of melondialdehyde (MDA) formed/minutes using extinction coefficient 1.56×10^5 as described by Utely et. al.

Calculation :

Lipid Peroxidation was calculated using the farmulla.

$$\textbf{Nanomoles of MDA formed/min} = \frac{\textbf{O.Dx1000 x 1000 x 1000 x total vol x 8}}{\textbf{1.56 x 100000 x 1000 x 10}}$$

Where,

MDA = Melondialdehyde,

O.D = Changes of optical density,

Statistical Analysis :

The data were analysed using student's "t" test significant difference between means of treated and control groups were calculated and "P" values were obtained.

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